Functional Ecology

British Ecological Society

Functional Ecology 2010, **24**, 898–908 doi: 10.1111/j.1365-2435.2010.01707.x

Evidence for a temperature acclimation mechanism in bacteria: an empirical test of a membrane-mediated trade-off

Edward K. Hall*[,1,2], Gabriel A. Singer[2], Martin J. Kainz[3] and Jay T. Lennon[1,4]

[1]*W.K. Kellogg Biological Station, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060, USA;* [2]*Department of Limnology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria;* [3]*Inter-University Center for Aquatic Ecosystems Research WasserCluster Lunz, Dr. Carl Kupelwieser Promenade 5, A-3293 Lunz am See, Austria; and* [4]*Department of Microbiology and Molecular Genetics Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060, USA*

Summary

1. Shifts in bacterial community composition along temporal and spatial temperature gradients occur in a wide range of habitats and have potentially important implications for ecosystem functioning. However, it is often challenging to empirically link an adaptation or acclimation that defines environmental niche or biogeography with a quantifiable phenotype, especially in micro-organisms.

2. Here we evaluate a possible mechanistic explanation for shifts in bacterioplankton community composition in response to temperature by testing a previously hypothesized membrane mediated trade-off between resource acquisition and respiratory costs.

3. We isolated two strains of *Flavobacterium* sp. at two temperatures (cold isolate and warm isolate) from the epilimnion of a small temperate lake in North Central Minnesota.

4. Compared with the cold isolate the warm isolate had higher growth rate, higher carrying capacity, lower lag time and lower respiration at the high temperature and lower phosphorus uptake at the low temperature. We also observed significant differences in membrane lipid composition between isolates and between environments that were consistent with adjustments necessary to maintain membrane fluidity at different temperatures.

5. Our results suggest that temperature acclimation in planktonic bacteria is, in part, a resource-dependent membrane-facilitated phenomenon. This study provides an explicit example of how a quantifiable phenotype can be linked through physiology to competitive ability and environmental niche.

Key-words: acclimation, adaptation, bacteria, competitive trade-offs, membrane composition, resource acquisition, respiration, temperature

Introduction

The effect of temperature on bacterial community metabolism is multifaceted, containing physical, physiological and ecological components. Studies that evaluate the response of micro-organisms from a broad range of habitats often find no clear patterns between environmental temperature and community physiology (Rivkin, Anderson & Lajzerowicz 1996). One reason for this is that the effect of temperature on bacterial growth is resource dependent, leading to similar levels of bacterial growth in low-temperature/high-resource environments as in high-temperature/low-resource environments (Pomeroy & Wiebe 2001). In addition, bacterial communities appear to be functionally adapted or acclimated to *in situ* temperature. Lacustrine bacterioplankton communities have maximal nutrient use efficiency near *in situ* temperature (Hall *et al.* 2009), and pelagic marine bacterioplankton have maximal growth near *in situ* temperature, across vertical and latitudinal spatial temperature gradients (Fuhrman & Azam 1983; Simon, Glockner & Amann 1999). Such optimization in community physiology with *in situ* temperature suggests that recurring seasonal differences in bacterial community composition in marine (Fuhrman *et al.* 2006), lentic (Shade *et al.* 2007), and lotic (Crump & Hobbie 2005; Hullar, Kaplan & Stahl 2006) ecosystems may be driven to a large degree by temperature. However, these differences in community structure and function across temperature gradients have yet to be clearly linked through a

*Correspondence author. E-mail: ed.hall@univie.ac.at

temperature-dependent species-specific mechanism. A mechanistic understanding of these dynamics would provide a better understanding of the controls on temporal and spatial community dynamics and may even elucidate how those dynamics affect bacterially mediated biogeochemical cycles.

A purely mathematical approach linked the physiological and ecological effects of temperature on microbial communities by demonstrating how temperature-dependent physiology could lead to differences in competitive ability (Hall, Neuhauser & Cotner 2008). The model included a trade-off between the ability to take-up resources at low temperatures and limit respiratory costs at high temperatures. This trade-off was sufficient to allow for competitive exclusion, and thus shifts in community composition, over a temperature gradient. The physiological rationale for such a trade-off is consistent with a previously proposed mechanism to explain differences in specific affinity for resources with changing temperatures (Nedwell 1999) and requires membrane lipid composition to be a species-specific phenotype with limited plasticity.

The rationale for the hypothesized trade-off is built on a foundation of fundamental relationships between bacterial membrane composition, thermal properties of membrane lipids and bacterial physiology. In bacteria, the viscosity of the semi-fluid membrane lipid mosaic must be maintained to allow for proper function of transmembrane proteins while simultaneously maintaining proton motive force (Sinensky 1974; Kaneda 1991; van de Vossenberg *et al*. 1999). At low temperatures, membranes can be too rigid and prevent the efficient function of transmembrane proteins, essential for resource acquisition. However, in bacteria the cellular membrane is also used to create an electro-chemical gradient (proton motive force), which drives synthesis of ATP as protons move down the proton gradient into the cell. A membrane that is too fluid can be 'leaky', allowing protons to return by non-energy-yielding pathways (i.e. pathways that are not linked to an ATPase). Without the ability to adjust membrane composition bacteria can compensate for leaky membranes by pumping more protons across the cellular membrane, i.e. increasing respiration (van de Vossenberg *et al*. 1999). Therefore, in order to optimize physiology it is essential to maintain membrane fluidity within a defined range of viscosity, which can be challenging in a variable temperature environment.

The overall viscosity of a bacterial membrane at a given temperature is determined by the relative proportion of its constituent fatty acids (Sinensky 1974). Bacterial membranes consist of a broad diversity of fatty acids, each with distinct thermal properties (Knothe & Dunn 2009). The differences in the thermal properties of lipid membranes can be attributed to the number of double bonds (Farkas *et al*. 1994), and side chains or branches (Kaneda 1991) in the fatty acid tails of the membrane lipids. All else being equal, the melting point of lipids decreases from saturated fatty acids (SAFA) > monounsaturated fatty acids (MUFA) > polyunsaturated fatty acids (PUFA), while the presence of branched fatty acid tails also decreases the melting temperature of a membrane (Silvius 1982). Under nutrient-replete conditions, bacteria can alter their membrane lipid composition to acclimate to changing temperatures (van de Vossenberg *et al*. 1999), a process known as homeoviscous adaptation (Sinensky 1974). However, the synthesis of *de novo* lipids is energetically costly (White 2000) and under oligotrophic conditions lipid composition may be a phenotype with limited plasticity. Therefore, micro-organisms living in oligotrophic environments, the rule rather than the exception, should have a relatively static membrane composition. In the absence of a plastic membrane phenotype, organisms are out-competed for limiting resources, resulting in competitive exclusion and shifts in community composition across thermal gradients (Hall, Neuhauser & Cotner 2008). One would then hypothesize cold environments to be dominated by organisms rich in MUFA or branched chain fatty acids while warmer environments would likely be dominated by organisms with SAFA (PUFA, while not undocumented, are rare in bacteria; Kaneda 1991). These temperature-dependent physical properties of bacterial membranes and the hypothesized trade-off between resource uptake and respiration comprise a complete mechanistic framework linking phenotype, physiology and competitive ability and ultimately result in communities that are acclimated and/or adapted to *in situ* temperature.

Adaptation and acclimation are often used to mean different things in different portions of the scientific literature. Here we define acclimation as phenotypic plasticity in a single isolate across environmental space (temperature and resource) during the course of our experiments (i.e. hours to weeks). Conversely, a static phenotype that is consistently different between isolates and consistent with an advantage at a given temperature (as discussed above), but with limited plasticity over environmental space we term adaptation. These terms need not be mutually exclusive or even partially exclusive. For example, adaptation could also be the range of acclimation one isolate is capable of relative to another. Regardless, by explicitly defining each term here we hope to avoid confusion associated with our use of these terms and how they are used in other portions of the scientific literature. Following these definitions, we evaluate how the composition of fatty acids in bacterial membranes influences acclimation, and to some extent adaptation, to temperature, and how these physiological differences combine to define an organism's thermal niche.

We designed this study to test three specific hypotheses derived from the aforementioned theoretical work using two *Flavobacterium* sp. strains isolated at two different temperatures. First we hypothesized that the strain isolated at 4 °C (cold isolate = CI) would have higher resource uptake at lower temperatures relative to the strain isolated at 22 °C (warm isolate = WI). Secondly, we hypothesized that the WI would have lower respiration at the warmer temperature compared with the CI. Thirdly, we hypothesized that differences in the composition of cellular membrane fatty acids would reflect each isolate's temperature-dependent physiology in a manner related to known thermo-physical properties of specific fatty acids (i.e. the CI should, on average, have a higher proportion of MUFA or branched SAFA relative to the WI). Finally, to evaluate if the availability of resources

affects the plasticity of any of these phenotypes (Pomeroy & Wiebe 2001), we addressed each of these hypotheses at high and low resource phosphorus (P) levels.

Materials and methods

ISOLATION AND IDENTIFICATION

Heterotrophic bacteria were isolated from the surface water (~23 °C at the time of sampling) of Lake Itasca (Clearwater County, Minnesota, MN, USA). Lake Itasca is mesotrophic (chlorophyll *a* ~11 μg L^{-1}) with a surface area of $4{\cdot}4 \times 10^6$ m^2 and a maximum depth of 12 m. In July 2006, we plated dilutions of surface water samples onto Luria Broth agar plates, which were then incubated in the dark at either 4 or 22 °C and monitored daily for colony formation. Single colony isolates were transferred onto new agar plates two to three times to help ensure that we obtained isogenic lines. Genomic DNA was collected from single colonies by lysing the cells, which was followed by a purification step using a Fast DNA Spin Kit (Ghosh & LaPara 2007). The resulting DNA was used as template in PCR reactions targeting the 16S rRNA gene using primers 338F (5′-ACT CCT ACG GGA GGC AGC AG-3′) and 907R (5′-CCG TCA ATT CCT TTR AGT TT-3′) according to the conditions described in Ghosh & LaPara (2007). Sequencing was performed at the Advanced Genetic Analysis Center at the University of Minnesota using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). We determined the closest relatives of the bacterial isolates by conducting BLAST searches in the GenBank database (http://www.ncbi.nlm.nih.gov). Once identified the isolates were cryopreserved at −80 °C in 20% glycerol. For this study we chose two closely related taxa: one isolate from the 4 °C enrichment (CI) and one isolate from the 22 °C enrichment (WI). BLAST results revealed that both isolates were most closely related to *Flavobacterium* (phylum Bacteroidetes). After NAST alignment, we found that the two *Flavobacterium* isolates were 99·5% similar based on sequence variation of the 16S rRNA gene (DeSantis *et al*. 2006).

EXPERIMENTAL DESIGN

We tested our hypotheses by growing the two heterotrophic bacterial isolates (WI and CI) in batch culture at two different temperatures (5 and 28 °C, representative of the annual temperature range in Lake Itasca) on a minimal media (Tanner 2005) with low (23 μmol P L^{-1}) and high (230 μmol P L^{-1}) P amendments (2 isolates × 2 temperatures × 2 resource P-levels). Cultures were grown in 250 mL flasks, in temperature-controlled (± 1 °C) shaking incubators (100 r.p.m.). We conducted growth curve experiments with both isolates at each temperature in order to define the relative differences in temperature-dependent growth between isolates. P-uptake was measured for each isolate at the colder temperature (5 °C) using a radio-labelled KPO_4 method (see below). Respiration rates were determined for each isolate and resource P-level at 28 °C, while membrane lipid composition was analysed from cultures of both isolates and all treatments.

GROWTH CURVES

Growth was measured as changes in optical density, at 450 nm (OD450) with a 96 well microplate reader (100 μL aliquots; Molecular Devices, Sunnydale, CA, USA). OD data were used to fit (least squares) the following growth model:

$$\mathrm{OD} = \frac{a}{1 + e^{=(t-L/(b))}}$$

where a is the carrying capacity, t is time, L is lag and b is the maximum growth rate (Lennon *et al*. 2007).

P-UPTAKE

^{33}P-KPO_4 was added with non-radioactive phosphorus (^{31}P-KPO_4) to each culture aliquot at five concentrations (5, 10, 50, 75 and 250 μmol L^{-1}). After 3 min incubations, we collected cells on 25 mm nitrocellulose filters (0·2 μm pore size) pre-rinsed with non-radioactive KPO_4 (1 mM), to minimize non-specific sorption of the radio-labelled $^{33}PO_4$ to the filter. After filtration, all filters were rinsed again with non-radioactive KPO_4 and subsequently with cold nanopure water and placed into 7 mL glass scintillation vials with 4 mL of scintillation cocktail. We used a Wallac (Waltham, MA, USA) 1409 scintillation counter to quantify counts per minute, which were converted to decays per minute based on the efficiency of an unquenched standard. P-uptake values were normalized for bacterial biomass in each aliquot and fit to a power function: P-uptake $= a \times P^b$, where a and b are coefficients fit to the data to minimize residuals in a least square fit and P is resource phosphorus in μmol L^{-1}.

RESPIRATION MEASUREMENTS

We measured respiration rates of each isolate at each resource P-level at 28 °C by placing 5 mL aliquots of culture in a 10 mL glass respiration chamber, within a closed re-circulating water jacket, continuously supplied by a temperature-controlled water bath (StrathKelvin Instruments Ltd, North Lankshire, Scotland). Changes in the dissolved oxygen concentration within the culture aliquot were measured using Strathkelvin 1302 oxygen electrodes coupled with a StrathKelvin 782 oxygen meter. Respiration rate was estimated in duplicate as the slope of the decrease in oxygen over time using simple linear regression. Respiration rate was then divided by biomass of the aliquot and reported as biomass specific respiration (h^{-1}).

BIOMASS ESTIMATES

To account for differences in biomass between treatments, P-uptake and respiration were normalized and reported per μmol of biomass carbon (C) in each sample. At the time of P-uptake and respiration measurements, OD 450 was also measured. OD measurements were converted to bacterial abundance (BA) from an empirically derived relationship between BA and OD. Samples for BA were preserved with formalin (2% final concentration). Cells were stained with Acridine Orange (Hobbie, Daley & Jasper 1977) and counted using a Zeiss Axioplan epifluorescent microscope (Zeiss, Oberkochen, Germany). Cell width and length were measured for at least 300 cells per slide with Axioplan image analysis software. We calculated mean cell biovolumes using the equation: $\pi/4 \times W^2 \times (L - W/3)$ and converted to μmol carbon using $5{\cdot}6 \times 10^{-13}$ g C μm^{-3} (Bratbak 1985).

LIPID AND FATTY ACID ANALYSIS

We collected bacterial biomass for lipid analysis by filtering 10–50 mL of cell culture through pre-combusted GF/F filters

(Whatman). Filters were placed in cryovials and stored at −80 °C until analysis. After freeze-drying, total lipids were extracted using chloroform–methanol–water mixture, 4 : 2 : 1 volume to volume (Kainz, Arts & Mazumder 2004). We analysed fatty acids as methyl esters (FAME) using a gas chromatograph (Trace GC Ultra; THERMO, Waltham, MA, USA) equipped with a flame ionization detector. The methyl esters were prepared by trans-esterifying the lipid extract in a H_2SO_4 : CH_3OH solution (1 : 100, v/v). Samples were flushed with N_2, vortexed and incubated (16 h) at 50 °C in a water bath. After cooling, $KHCO_3$ (2%), isohexane : diethyl ether (5 mL; 1 : 1, v/v) and butylated hydroxyl toluene (50 μg) were added, and the sample shaken and centrifuged. Subsequently, the organic layer was transferred and the solvent evaporated under N_2. FAME were dissolved in isohexane and analysed on a Supelco™ (St. Louis, MO, USA) 2560 Capillary Column (100 m, 0·25 mm i.d., 0·2 μm film thickness) with helium as the carrier gas at a flow rate of 1 mL min^{-1}. FAME were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco™ #47885-U; and bacterial acid methyl ester mix, BAME, Supelco™ #47080-U).

STATISTICAL ANALYSES

We analysed each of the physiological characteristics (growth, respiration and P uptake) using maximum likelihood analysis (MLE) by simultaneously testing multiple models that included the factors resource (high vs. low resource P), temperature (5 vs. 28 °C) or isolate (WI vs. CI; Burnham & Anderson 2002). By comparing the Akaike Information Criterion (AIC) between models we were able to select the most parsimonious model with the best overall fit (Burnham & Anderson 2002). At a difference of less than two AIC units between models, the models were considered to be equivalent indicating no improvement of fit with the additional factor (Burnham & Anderson 2002). The parameters of the growth curve fits (lag, carrying capacity and growth rate) were independently evaluated for direct and indirect effects of temperature, resource P-level and isolate. Respiration was evaluated for effects of resource P-level and isolate, while P-uptake was only evaluated for difference between isolates. In each case we report the AIC score and the difference between the AIC score (delta AIC) for each model and the null model (i.e. a model that considers none of the factors).

Differences in membrane fatty acid composition between treatments were evaluated by computing pairwise Bray–Curtis dissimilarities (Bray & Curtis 1957; Legendre & Legendre 2003) between individual replicate samples from arcsine-square root-transformed proportional lipid data. The triangular dissimilarity (distance) matrix then served as input for nonparametric MANOVA (i.e. PERMANOVA) which assesses between-group distances over within-group distances by computation of pseudo-*F*-values and permutational assessment of significance (Anderson 2001; McArdle & Anderson 2001). Homogeneity of multivariate dispersion among groups was assessed similarly by permutational analysis (Anderson 2006). We tested for main effects and interaction effects of temperature, resource P-level and isolates on multivariate fatty acid composition. The Bray–Curtis dissimilarity matrix was further subjected to cluster analysis and non-metric multidimensional scaling (NMDS). Contributions of the various fatty acids to the dissimilarity between any two replicates can be computed from an additive partitioning of Bray–Curtis dissimilarity (Clarke 1993). When these contributions are calculated between all pairs of replicates belonging to two different groups, then fatty acids with a large ratio of average : standard deviation of contributions to dissimilarity are the best discriminators between two groups. These computations were made for the two groups defined by one of the three factors (temperature, resource P-level or isolate) while restricting the formation of replicate pairs within the same levels of the remaining two factors. Fatty acids identified as important discriminators were then fit onto the NMDS ordination to show direction and strength of the discriminating power of each. Individual fatty acids were also summed to yield the proportion of MUFA (from total identified fatty acids) and the proportion of branched SAFA (from all SAFA). As only minimal PUFA were identified, the proportion of SAFA (from total identified fatty acids) is a direct transformation from the proportion of MUFA and further tests on the proportion of SAFA or PUFA were redundant. To keep the nonparametrical nature of fatty acid data analyses, comparisons between resource P-levels, temperature treatments or isolates were made using Mann–Whitney *U*-tests without accounting for interactive effects. All statistical analyses were performed in the statistical software R using packages bbmle, MASS and vegan (R Development Core Team 2005).

Results

GROWTH, P-UPTAKE AND RESPIRATION

The growth dynamics of each isolate reflected the temperature at which the strain was isolated (Fig. 1). While at the colder temperature (5 °C) there were no clear differences between growth characteristics of the CI and WI (Fig. 1b,d) at 28 °C the CI had a longer lag phase and lower carrying capacity than the WI (Fig. 1a,c). Differences in growth characteristics between isolates at 28 °C were confirmed by MLE analyses. For the model parameters 'lag phase' and 'growth rate' addition of the factor 'isolate' to the model improved the fit to the data (Table 1). Including a 'temperature × isolate' interaction improved model fit for all three growth parameters, indicating a strong difference between each isolate's growth dynamics between temperatures, which was most pronounced for the lag phase parameter (Table 1).

We evaluated the ability of each isolate to take up P at 5 °C by plotting P uptake rate as a function of resource P (Fig. 2). The CI had higher P-uptake than the WI at each of the five resource levels, consistent with our hypothesis (Fig. 2). The relationship between uptake rates and resource P for each isolate was best described by a power function which fit significantly to each data set ($p < 0{\cdot}01$, for both). Again, MLE analysis indicated that the model including an isolate effect fit better to the data than a model-ignoring isolate effect (Table 2). However, delta AIC was less than two AIC units and therefore, while the differences in P uptake were in the hypothesized direction the null model fit the data as well as the model-including the isolate effect (Table 2).

Isolates also differed in specific respiration rates in the hypothesized direction. At the warmer temperature (28 °C), respiration rate was higher for the CI than for the WI in both the low and high P treatments (Fig. 3). This result was true for cultures sampled in middle exponential phase (∼24 h, Fig. 3a) and late exponential phase (∼36 h, Fig. 3b) and was confirmed by MLE with delta AIC score ∼6 AIC units between the null model and the model that included the factor 'isolate' (Table 3).

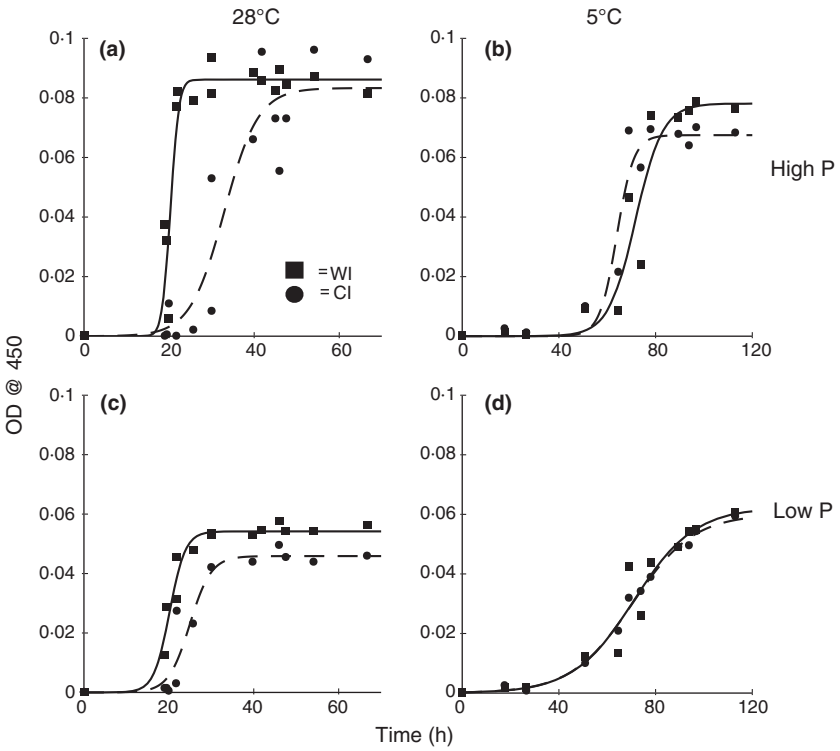

Fig. 1. Growth of the warm isolate (WI) and cold isolate at 28 °C and 5 °C at high and low resource P-levels in replicate. Each line represents a least squares fit of the growth model described in the text. The WI had consistently shorter lag phase and a higher carrying capacity in the 28 °C incubation.

Table 1. Results of MLE analysis of the growth characteristics. Given are differences of AIC scores (delta AIC) between the null model [sigmoidal function with lag, carrying capacity (*a*) and growth rate (*b*), no factors included as predictors] and models including various predictor terms. In addition, we report AIC weights (weight), which can be interpreted as the probability of each model being the best model given the candidate set of models. All models including isolate or an interaction with isolate, with the exception of the isolate-only model for carrying capacity, improved the model fit (highlighted in bold)

Model description	Delta AIC (lag)	Weight (lag)	Delta AIC (*a*)	Weight (*a*)	Delta AIC (*b*)	Weight (*b*)	d.f.
Null model	0·00	0·00	0·00	0	0·00	0·00	4
Temperature	13·83	0·00	23·59	0	3·97	0·02	5
P	1·21	0·00	17·88	0	0·64	0·00	5
Isolate	**15·93**	**0·00**	**1·64**	**0**	**3·63**	**0·01**	**5**
Temperature × P	12·19	0·00	47·33	0·99	9·83	0·30	7
Temperature × isolate	**76·28**	**1·00**	**33·00**	**0·00**	**11·40**	**0·65**	**7**
P × isolate	**13·15**	**0·00**	**16·58**	**0**	**3·46**	**0·01**	**7**

MLE, maximum likelihood analysis; AIC, Akaike Information Criterion.

Analysis of membrane fatty acid composition indicated each isolate had unique membrane composition but also demonstrated phenotypic plasticity across the experimental temperature and resource gradient (Table 4). In total, we identified 17 individual fatty acids in the various treatments: four MUFA and 11 SAFA of which four were branched, Linoleic acid (18:2n-6) was the only PUFA found in either isolate. In a cluster analysis lipid composition of each treatment grouped primarily by temperature, then by either isolate or resource level with only one misclassification (Fig. 4). At the colder temperature, treatments separated first by resource and then by isolate, while at the warmer temperature the treatments separated first by isolate and then by resource (Fig. 4). For the CI grown at 28 °C, two replicates each from the low P treatment grouped separately from all other treatments (Fig. 4), and were therefore excluded from further analyses.

In general, organisms grown at 5 °C had significantly more MUFA, and fewer SAFA than organisms grown at 28 °C ($z = 4{\cdot}87$, $P < 0{\cdot}001$), as hypothesized. Also, organisms grown at 5 °C had a higher proportion of branched SAFA ($z = 3{\cdot}96$, $P < 0{\cdot}001$) than organisms grown at 28 °C. When temperature treatments and isolates were combined, P had no effect on either the proportion of MUFA or SAFA ($z = 1{\cdot}59$, $P = 0{\cdot}11$), or the proportion of branched SAFA ($z = 1{\cdot}74$, $P = 0{\cdot}08$). Similarly, when temperature and P treatments were combined, there were no significant differences in the proportion of MUFA (or SAFA) between the two isolates ($z = 0{\cdot}52$, $P = 0{\cdot}60$). Rather, the majority of the differences between isolates could be attributed to differences in the proportion of SAFA and branched SAFA between the two isolates with the CI having a significantly higher percentage of branched SAFA than the WI ($z = 2{\cdot}33$, $P < 0{\cdot}05$). Thus, organisms shifted the composition of the

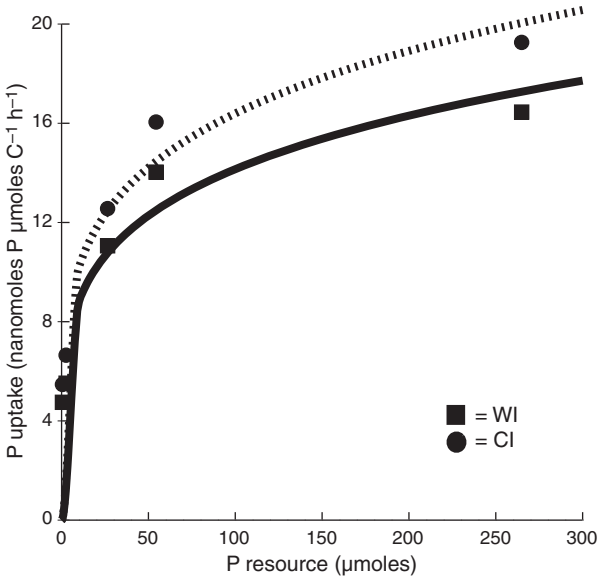

Fig. 2. Biomass specific P uptake rate for each isolate at 5 °C. The cold isolate (CI) had higher biomass specific P-uptake at each of five levels of resource P. Each curve represents a significant least squares fit of the power model for each isolate, described in the text for each isolate. Maximum likelihood analysis indicates that the uptake curves are not statistically distinguishable from each other.

Table 2. Results of MLE analysis of P uptake for each isolate from the low resource P culture and 5 °C incubation. Given are AIC scores of models (power function including coefficients *a* and *b* as defined in the text) with various predictor terms (e.g. isolate), difference (delta AIC) of AIC scores to the null model (no factors included), and the AIC weights, as described in the legend to Table 1

Model description	AIC	Delta AIC	d.f.	Weight
Null model	0·94	0	3	0·17
a coded by isolate	−0·73	1·67	4	0·39
b coded by isolate	−0·24	1·18	4	0·30
a and *b* coded by isolate	1·26	−0·32	5	0·14

MLE, maximum likelihood analysis; AIC, Akaike Information Criterion.

membrane fatty acids between temperature and resource treatments; however, within a treatment there were also differences between isolates. In each case shifts in lipid composition were consistent with adjusting membrane composition to maintain membrane fluidity.

Table 3. Results of MLE analysis of respiration at 28 °C. Given are AIC scores of models with various predictor terms, difference (delta AIC) in AIC scores to the null model (mean respiration rate, no factors included), and the AIC weights, as described in the legend to Table 1

Model description	AIC	Delta AIC	d.f.	Weight
Null model	94·82	0	2	0·02
Resource P-level	96·35	−1·52	3	0·01
Isolate	88·84	5·99	3	0·46
P + isolate	89·47	5·36	4	0·34
P × isolate	90·90	3·93	5	0·17

MLE, maximum likelihood analysis; AIC, Akaike Information Criterion.

We further evaluated the lipid data to understand how specific fatty acids affected the differentiation between temperature, resource P-level and isolate treatments. In general there were highly significant main effects of temperature (PERMANOVA, $F_{1,20} = 91{\cdot}48$, $P < 0{\cdot}001$), P ($F_{1,20} = 26{\cdot}95$, $P < 0{\cdot}001$) and isolate ($F_{1,20} = 22{\cdot}31$, $P < 0{\cdot}001$) on fatty acid composition. A significant temperature × P interaction ($F_{1,20} = 6{\cdot}92$, $P < 0{\cdot}01$) prompted us to run separate two-way analyses for each level of resource P. Interestingly, there was a stronger effect of temperature at the high resource P-level ($F_{1,12} = 79{\cdot}82$, $P < 0{\cdot}001$) than at the low resource P-level ($F_{1,8} = 22{\cdot}93$, $P < 0{\cdot}001$). These effects became visible in the NMDS-derived ordination where all eight treatments separated independently in multivariate space (Fig. 5). By additive partitioning of the dissimilarity we were able to clearly identify which fatty acids best discriminated between temperature treatments, resource P-levels and isolates (Table 5). For example, 16:1n-7 and 16:0 were good discriminators between temperature groups with cold treatments having relatively more 16:1n-7 and less 16:0, compared with the cultures grown at 28 °C (Table 4, Fig. 5). Similarly, i15:0 and 15:0 were the best discriminators for individual isolates (Table 5) with the CI having relatively more i15:0 and less 15:0 when compared with the WI (Table 4, Fig. 5). For each treatment we report the relative influence of each fatty acid on differentiating between temperature, resource P-level and isolate groups (Table 5).

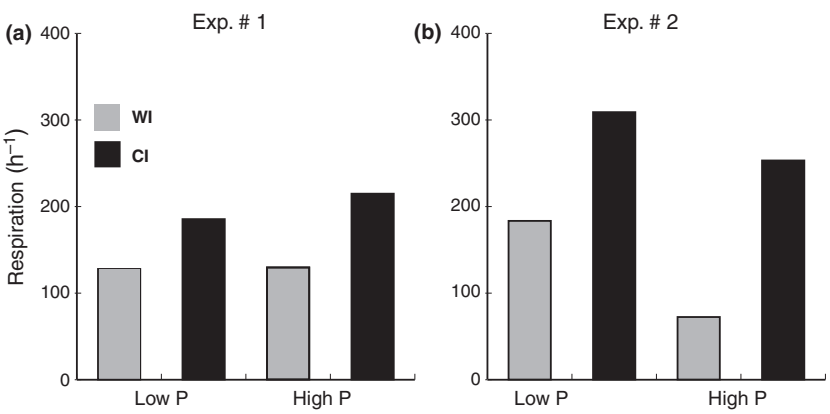

Fig. 3. Biomass specific respiration rate at 28 °C. The warm isolate had lower specific respiration (h^{-1}) for each resource treatment in each experiment. Experiment 1 and experiment 2 were conducted during early and late exponential growth, respectively, as described in the text.

Table 4. Mean proportion (with standard deviation) of each of the nine most influential lipids (as defined by the ratio of average : standard deviation of the contribution to Bray–Curtis similarities in determining differences between, temperature, resource P-level and isolate treatments). Iso (i) and anteiso (ai) refer to a fatty acid with a single methyl branch located two or three carbons away from the terminal methyl group respectively

Isolate	P	Temp (°C)	ai15:0	i15:0	15:0	i16:0	16:0	16:1n-7	i17:0	16:9,10Δ
WI	Low	6	28·1 (2·4)	10·0 (1·9)	3·5 (0·5)	1·5 (0·4)	3·9 (1·5)	41·4 (2·4)	0·1 (0·1)	0·6 (0·1)
CI	Low	6	28·1 (2·2)	18·5 (3·7)	1·6 (0·4)	1·7 (0·28)	3·4 (1·3)	36·4 (1·2)	0·1 (0·1)	0·2 (0·1)
WI	High	6	18·4 (1·4)	9·7 (2·2)	5·6 (0·7)	2·8 (0·3)	6·4 (0·2)	49·9 (0·4)	0·1 (0·1)	0·6 (0·04)
CI	High	6	18·9 (0·2)	16·1 (3·0)	3·1 (0·8)	2·6 (0·1)	4·9 (0·2)	47·8 (2·1)	0·1 (0·1)	0·3 (0·1)
WI	Low	28	23·4 (15·5)	4·2 (1·0)	4·1 (2·2)	1·2 (0·7)	9·2 (6·1)	20·0 (12·1)	1·0 (0·6)	0·7 (0·4)
CI	Low	28	15·0 (19·9)	27·8 (26·6)	1·9 (1·0)	8·8 (9·3)	10·5 (4·4)	9·7 (12·3)	1·1 (0·4)	0·2 (0·2)
WI	High	28	29·3 (6·4)	12·9 (9·2)	4·9 (2·6)	4·5 (3·2)	12·0 (5·0)	19·1 (4·8)	1·6 (0·8)	0·6 (0·2)
CI	High	28	22·7 (11·0)	8·6 (1·90)	3·0 (0·7)	1·9 (0·9)	12·0 (5·1)	22·9 (12·8)	1·0 (0·1)	1·2 (1·3)

16:9,10Δ, cis-9,10-methylenehexadecanoic acid (CAS no. 4675-60-9); WI, warm isolate; CI, cold isolate.

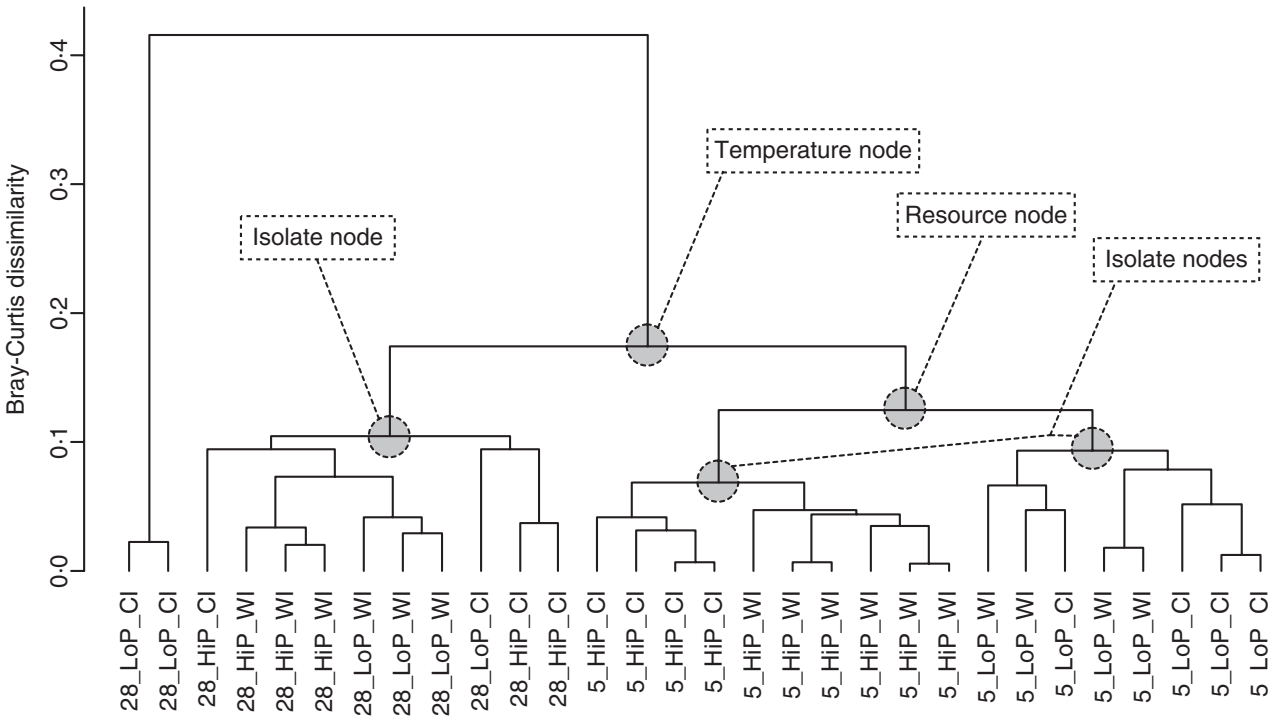

Fig. 4. A cluster analysis of lipid composition using Bray–Curtis dissimilarity on arcsine-square root-transformed proportional lipid data as a distance measure and unweighted pair-group average clustering. Replicates are coded by 'temperature-resource-isolate' with the levels 5 and 28 °C (incubation temperature), LoP and HiP (low and high resource P-level respectively) resource, and CI and WI (cold and warm isolate). The two cases on the far left sorted independently of all other treatments and were excluded from further analysis.

Discussion

The results presented here are consistent with the hypothesized model of temperature acclimation in freshwater bacteria as a resource-dependent membrane-facilitated phenomenon. Each isolate had well-defined physiological differences between the two temperatures. The WI grew better at the warm temperature than did the CI. Also, the CI had higher, although not significantly different, P-uptake at the colder temperature and higher respiration at the warmer temperature than the WI, consistent with the hypothesized trade-off between resource uptake and respiration (Hall, Neuhauser & Cotner 2008). In addition to these physiological differences, the fatty acid composition was significantly different between isolates, and changed within a given isolate with both temperature and resource treatments. In each case, shifts in fatty acid composition were qualitatively consistent with changes predicted to maintain membrane fluidity with changing temperatures and thus indicative of acclimation or adaptation to a given temperature.

Our data suggest that adjusting the relative quantity of branched fatty acids was the primary mechanism these freshwater isolates used to adjust to temperature. We expected the CI to have a membrane composition consisting of fatty acids with lower melting points, which would allow for less rigidity at the lower temperatures (consistent with higher uptake

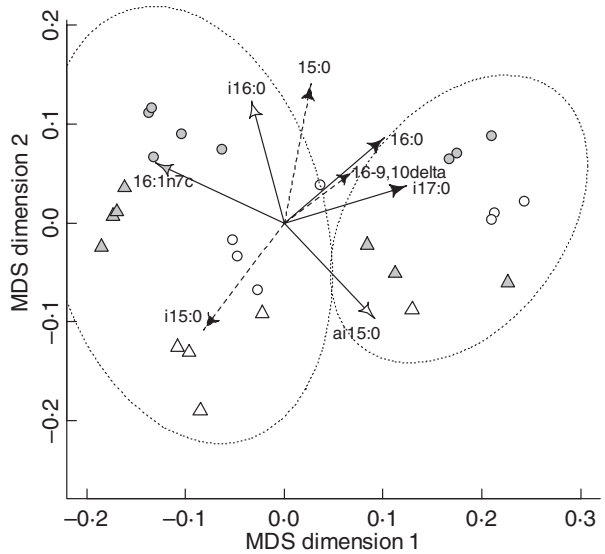

Fig. 5. Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity on arcsine-square root-transformed proportional lipid data. Kruskal's stress is 0·077. Dashed ellipses (95% intervals around centroids) perfectly separate the two levels of the temperature treatment (5 °C = left ellipse; 28 °C, = right ellipse). Circles and triangles represent the warm and cold isolate, while white and grey symbols indicate low and high P levels respectively. Vectors show direction and strength of gradient (correlation) of selected fatty acids with the ordination. The fatty acids shown as vectors were the best discriminators (see Table 5) between temperature (black and grey arrows), resource (white and grey arrows) and isolate (black dashed arrow) groups. Representation as vectors was chosen as the various lipids changed monotonically and approximately linearly over ordination space.

rates) and result in higher fluidity at the higher temperatures (consistent with higher respiration). While there were no clear differences between the relative proportion of MUFA and SAFA between isolates, there were consistent differences in the proportion of branched and unbranched SAFA in the predicted direction (i.e. CI had more branched SAFA than the WI). Strategies of lipid membrane adaptation to cold temperature are known for planktonic invertebrates (Farkas 1979) and fish (Arts & Kohler 2009), which show increases in the number of double bonds in their membrane lipids, i.e. more MUFA and PUFA relative to SAFA. Bacteria have only rarely been shown to produce PUFA (Nichols, Nichols & McMeekin 1993; Nichols *et al*. 1997) and are thought to mainly use branched fatty acids to adjust membrane fluidity (Kaneda 1991). Previous studies have noted the importance of relative shifts in branched chain fatty acids as an important mechanism in regulating membrane fluidity in bacteria, where iso-acyl chains (e.g. i15:0) were shown to have lower melting points (18–28 °C) than their saturated acyl-chain counterparts (Silvius 1982).

From the analysis presented here it is clear that certain fatty acids were part of a physiological shift, triggered by changes in temperature and resources, while others were more rigidly associated with each isolate. For both isolates, the proportions of the branched fatty acid i15:0 and the unbranched fatty acid 15:0 changed relatively little across the resource levels (Table 4) and were the two best discriminators between isolates (Table 5). Specifically, the CI consistently had higher proportion of i15:0 relative to the WI, which had a higher proportion of 15:0 (Fig. 5). The relative proportions of these fatty acids changed between temperature and P treatments, but within a given environment each isolate was significantly different from the other and maintained relative differences in these fatty acids (Fig. 5). It is difficult to directly link the differences in the fatty acid composition to physiological performance. However, the relative differences in 15:0 and i15:0 between the two isolates were consistent with a warm-adapted

Table 5. Contribution of the various lipids to Bray–Curtis dissimilarity between groups defined by the factors temperature (5 °C vs. 28 °C), resource (high vs. low resource P) and isolate (warm vs. cold isolate). Given are percentage contribution (and ratio average : standard deviation of contribution). High ratio average : standard deviation indicates strong discriminatory power between groups. Best three discriminators for each factor are printed in bold and shown as vectors in Fig. 5. The last column reports average proportion (and standard deviation) of lipids across all treatments. For clarity three lipids of minor importance (ratio average : standard deviation < 1 for all factors) are excluded from the table

	Temperature Avg. diss. = 0·17	Resource Avg. diss. = 0·11	Isolate Avg. diss. = 0·08	Avg. proportion (±SD)
16:1n-7	**22·18% (2·89)**	**14·16% (2·59)**	8·69% (1·18)	35·25 (4·59)
16:0	**14·49% (4·32)**	8·55% (1·67)	7·01% (1·62)	8·19 (8·96)
ai15:0	11·43% (2·03)	**15·9% (2·16)**	5·72% (0·97)	24·37 (0·7)
i17:0	**9·38% (4·15)**	2·55% (1·06)	3·38% (1·02)	0·61 (3·42)
18:1n-9	8·19% (1·08)	9·47% (1·22)	8·04% (0·72)	4·57 (11·08)
i15:0	8·04% (1·36)	7·38% (1·57)	**19·2% (1·81)**	13·64 (0·68)
18:2n-6	3·95% (1·66)	4·12% (1·66)	3·97% (1·07)	1·21 (1)
18:0	3·73% (1·64)	6·09% (1·28)	4·54% (0·97)	1·69 (0·57)
17:0	2·97% (1·57)	4·55% (1·52)	4·07% (1·18)	0·94 (1·77)
15:0	2·75% (1·44)	7·28% (1·86)	**13·1% (2·95)**	3·87 (0·55)
16:9,10Δ	2·61% (0·77)	1·6% (0·62)	**6·02% (1·78)**	0·59 (0·24)
12:0	2·26% (1·61)	3·13% (1·63)	2·53% (1·11)	0·47 (0·37)
14:0	2·11% (2·39)	1·27% (1·18)	3·16% (1·64)	1·46 (3·81)
i16:0	2·06% (1·21)	**6·56% (2·94)**	3·81% (1·19)	3·06 (0·1)
Sum of accounted diss. or total lipids	96·15%	92·61%	93·24%	99·91%

WI and a cold-adapted CI, suggesting differences in these fatty acids were an adaptation to a specific thermal regime. On the other hand, 16:1n-7 (more in the cold treatment) and 16:0 (more in the warm treatment) were among the best discriminators between temperature treatments and thus were indicative of fatty acids that play an important role in physiological acclimation in these isolates (Table 5, Fig. 5). Interestingly, resource P-level significantly modified the temperature acclimation response as indicated by the significant interaction between resource P-level and temperature (PERMANOVA). The effect of resource P-level on membrane composition between temperature environments can be expressed in terms of a multivariate distance measure (i.e. the averaged distance between replicates belonging to either of two temperature groups). The average Bray–Curtis dissimilarity between the 5 and 28 °C treatments was 0·145 for the low resource P-level vs. 0·173 for the high resource P-level with non-overlapping 95% confidence intervals of 0·126–0·159 vs. 0·161–0·192 generated by bootstrapping. Thus, isolates grown under high P resource showed a significantly stronger temperature acclimation response than isolates grown in the low P resource environment. This is an important result as it empirically demonstrates that resource availability can constrain phenotypes that are critical to temperature acclimation. While interactions between temperature and resource have been well documented in bacterioplankton (Pomeroy & Wiebe 2001), to our knowledge a resource-dependent phenotype constraint, with respect to temperature acclimation, has not been previously considered. This adds yet another level of complexity to the physiological response to temperature by micro-organisms in the environment. Without the ability to change membrane composition, species become less competitive for limiting resources (e.g. P) and will be outcompeted by other species with more appropriate temperature-specific phenotypes, resulting in shifts in community composition (Hall, Neuhauser & Cotner 2008).

Differences in community composition are normally evaluated at a relatively coarse phylogenetic resolution, with 97% similarity in rRNA sequence used as a common cut-off to group organisms within operational taxonomic units. In this study, we show evidence for significant phenotypic variability within two closely related isolates whose rRNA sequence places them at ~99·5% similarity. Additional studies have also shown ecological partitioning of closely related bacteria. A study of marine bacteria revealed distinct spatial partitioning of closely related members of the family Vibrionaceae (Hunt *et al.* 2008). More closely related to the results presented here, a separate study identified differences in thermal adaptation of freshwater bacterioplankton with highly similar 16S rRNA gene sequences isolated from aquatic ecosystems across a broad latitudinal range. Closely related strains isolated from tropical systems demonstrated physiological acclimation to warmer temperature while strains isolated from higher latitudes had higher growth rate at lower temperatures (Hahn & Pockl 2005). We also found genetically similar isolates with distinct temperature-dependent physiologies and membrane composition. However, in our study these organisms were isolated from the same ecosystem at the same time of the year. This suggests that thermal adaptation conveyed through differences in membrane fatty acid composition may be one aspect of 'hidden' phenotypic diversity that is present within genetically similar individuals. This result emphasizes the difficultly in linking community composition to function, as even closely related individuals appear to be of distinct ecotypes (Polz *et al.* 2006).

The mechanism proposed here might also be the underlying mechanism responsible for patterns of temperature-dependent microbial physiology observed in other habitats. Recently, acclimation and adaptation of soil microbial communities to temperature, specifically respiration, have received a good deal of attention (Balser & Wixon 2009; Bradford, Watts & Davies 2010; Rinnan *et al.* 2009). In one study, soil communities were allowed to acclimate to one of three temperatures for a total of 77 days and then respiration was measured in short incubations at all three temperatures. The soil communities from the lowest temperature incubation had the highest respiration response to increasing temperature, while those from the highest temperature decreased their respiration over the course of the incubation (Bradford, Watts & Davies 2010). High respiration at high temperature by the cold acclimated communities would be expected with acclimation or adaptation to lower temperature following the mechanisms discussed above. This result is also consistent with observations of community respiration in aquatic ecosystems, where winter communities showed higher respiration at the warm experimental temperatures than did summer communities (Hall & Cotner 2007). The study of soil communities focused on differences in thermal sensitivity of intracellular and extracellular enzymes as a mechanism for acclimation to temperature. Given the complexity in carbon substrates in terrestrial ecosystems this may indeed be a more important mechanism for acclimating to *in situ* temperature. However, the similarities in the community level responses (Hall & Cotner 2007; Bradford, Watts & Davies 2010) suggest that the acclimation mechanism proposed in this study may also be active in soil microbial communities and therefore merits further evaluation. Furthermore, because bacterial membranes are active sites for biochemical activity, shifts in lipid composition might affect rate processes in addition to the effects on proton permeability and resource transport discussed here (Sinensky 1974).

In summary, we used a system of two natural bacterial isolates to demonstrate the importance of membrane lipid composition in defining an organism's thermal niche. We showed how specific lipids were indicative of an acclimation response to changing temperature while others were more rigidly associated with one isolate or the other, and therefore consistent with an adaptive response to temperature. Furthermore, resource level clearly affected the acclimation response to temperature, demonstrating that resource availability can limit the range of phenotypic plasticity. Placed in the context of previous research (Shade *et al.* 2007; Hall, Neuhauser & Cotner 2008) this provides a complete mechanistic link between temperature-dependent physiology, com-

petitive ability and community structure. While there are a myriad of physiological and biochemical changes in microorganisms that accompany changes in temperature, the mechanism outlined here provides one possible explanation for reported seasonal and biogeographical patterns in bacterial community structure that have been correlated with differences in *in situ* temperature (Fuhrman & Azam 1983; Simon, Glockner & Amann 1999; Fuhrman *et al*. 2006; Hullar, Kaplan & Stahl 2006; Shade *et al*. 2007). Our study included two closely related isolates of the phylum Bacteroidetes, which has been identified as a significant component of cosmopolitan freshwater communities (Zwart *et al*. 2002). Furthermore, *Flavobacterium* isolates from both soils and freshwater ecosystems have been shown to contain a large proportion of the fatty acids that we found to be important components of temperature adaptation and acclimation in the isolates used in this study. Specifically i15:0 – one of the best discriminators between isolates – was consistently found to be in high relative abundance of environmental isolates of the genus *Flavobacterium* (Bernardet *et al*. 1996; Cousin, Päuker & Stackebrandt 2007; Kim, Kim & Cha 2009). However, it is important to note that while isolated from a temperate lake, these organisms were cultured at relatively high resource levels and may or may not have physiologies that are representative of environmental bacterial communities. Only studies that explicitly evaluate the link between lipid composition, temperature-dependent physiology and community composition for a broader range of bacterial taxa and of microbial communities under *in situ* environmental conditions will determine how widespread this mechanism is in natural microbial communities. Developing such a mechanistic understanding of how temperature affects natural microbial communities is essential to understanding the fundamental links between temperature, microbial biogeography and the biogeochemical function of the associated community.

Acknowledgements

This research was funded through a grant to E.K.H. and J.T.L. from the Center for Water Science at Michigan State University. The authors thank J. Watzke for lipid analysis, T. LaPara for rRNA sequencing of the isolates used in the experiments, and J. Cotner, A. Little and T. Scott for isolation and supply of the experimental strains. Earlier drafts of this manuscript were improved through discussions with M. Wallenstein, S. Taipale, J. Lau and three anonymous reviewers.

References

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32–46.

Anderson, M.J. (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, **62**, 245–253.

Arts, M.T. & Kohler, C.C. (2009) Health and condition in fish: the influence of lipids on membrane competency and immune response. *Lipids in Aquatic Ecosystems* (eds M.T. Arts, M.T. Brett & M.J. Kainz), pp. 237–256. Springer, New York.

Balser, T.C. & Wixon, D.L. (2009) Investigating biological control over soil carbon temperature sensitivity. *Global Change Biology*, **15**, 2935–2949.

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996) Cutting a Gordian knot: emended classification and description of the Genus *Flavobacterium,* emended description of the Family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, Cytophaga aquatilis Strohl and Tait 1978). *International Journal of Systematic Bacteriology*, **46**, 128–148.

Bradford, M.A., Watts, B.W. & Davies, C.A. (2010). Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology*, doi: 10.1111/j.1365-2486.2009.02040.x.

Bratbak, G. (1985) Bacterial biovolume and biomass estimations. *Applied and Environmental Microbiology*, **49**, 1488–1493.

Bray, R.J. & Curtis, J.T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, **27**, 325–349.

Burnham, K. & Anderson, D. (2002) *Model Selection and Multimodel Interference: A Practical Information-Theoretic Approach*. Springer, New York.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Cousin, S., Päuker, O. & Stackebrandt, E. (2007) *Flavobacterium aquindurnense* sp. nov. and *Flavobacterium hercynium* sp. nov., from a hard-water creek. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 243–249.

Crump, B.C. & Hobbie, J.E. (2005) Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnology & Oceanography*, **50**, 1718–1729.

DeSantis, T.Z., Hugenholtz, P., Keller, K., Brodie, E.L., Larsen, N., Piceno, M., Phan, R. & Andersen, G.L. (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. *Nucleic Acids Research*, **34**, W394–W399.

Farkas, T. (1979) Adaptation of fatty acid compositions to temperature – a study on planktonic crustaceans. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology*, **64**, 71–76.

Farkas, T., Dey, I., Buda, C. & Halver, J.E. (1994) Role of phospholipid molecular-species in maintaining lipid-membrane structure in response to temperature. *Biophysical Chemistry*, **50**, 147–155.

Fuhrman, J.A. & Azam, F. (1983) Adaptations of bacteria to marine subsurface waters studied by temperature response. *Marine Ecology Progress Series*, **13**, 95–98.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V. & Naeem, S. (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 13104–13109.

Ghosh, S. & LaPara, T. (2007) The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. *ISME Journal*, **1**, 191–203.

Hahn, M.W. & Pockl, M. (2005) Ecotypes of planktonic actinobacteria with identical 16S rRNA genes adapted to thermal niches in temperate, subtropical, and tropical freshwater habitats. *Applied and Environmental Microbiology*, **71**, 766–773.

Hall, E.K. & Cotner, J.B. (2007) Interactive effect of temperature and resources on carbon cycling by freshwater bacterioplankton communities. *Aquatic Microbial Ecology*, **49**, 35–45.

Hall, E.K., Neuhauser, C. & Cotner, J.B. (2008) Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. *ISME Journal*, **2**, 471–481.

Hall, E.K., Dzialowski, A.R., Stoxen, S.M. & Cotner, J.B. (2009) The effect of temperature on the coupling between phosphorus and growth in lacustrine bacterioplankton communities. *Limnology and Oceanography*, **54**, 880–889.

Hobbie, J.E., Daley, R.J. & Jasper, S. (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, **33**, 1225–1228.

Hullar, M.A.J., Kaplan, L.A. & Stahl, D.A. (2006) Recurring seasonal dynamics of microbial communities in stream habitats. *Applied and Environmental Microbiology*, **72**, 713–722.

Hunt, D.E., David, L., Gevers, D., Preheim, S.P., Alm, E.J. & Polz, M.F. (2008) Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science*, **320**, 1081–1085.

Kainz, M., Arts, M.T. & Mazumder, A. (2004) Essential fatty acids within the planktonic food web and its ecological role for higher trophic levels. *Limnology and Oceanography*, **49**, 1784–1793.

Kaneda, T. (1991) Iso- and anteiso-fatty acids in bacteria – biosynthesis, function, and taxonomic significance. *Microbiological Reviews*, **55**, 288–302.

Kim, J.-H., Kim, K.-Y. & Cha, C.-J. (2009) *Flavobacterium chungangense* sp. nov., isolated from a freshwater lake. *International Journal of Systematic and Evolutionary Microbiology*, **59**, 1754–1758.

Knothe, G. & Dunn, R.O. (2009) A comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. *Journal of the American Oil Chemists Society*, **86**, 843–856.

Legendre, L. & Legendre, P. (2003) Numerical ecology. *Developments in Environmental Modeling*, pp. 287, Elsevier Science, Amsterdam.

Lennon, J., Khatana, S., Marston, M. & Martiny, J. (2007) Is there a cost of virus resistance in marine cyanobacteria. *The ISME Journal*, **1**, 300–312.

McArdle, B.H. & Anderson, M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, **82**, 290–297.

Nedwell, D.B. (1999) Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. *FEMS Microbiology Ecology*, **30**, 101–111.

Nichols, D.S., Nichols, P.D. & McMeekin, T.A. (1993) Polyunsaturated fatty acids in antarctic bacteria. *Antarctic Science*, **5**, 149–160.

Nichols, D.S., Brown, J.L., Nichols, P.D. & McMeekin, T.A. (1997) Production of eicosapentaenoic and arachidonic acids by an antarctic bacterium: response to growth temperature. *FEMS Microbiology Letters*, **152**, 349–354.

Polz, M.F., Hunt, D.E., Preheim, S.P. & Weinrech, D.M. (2006) Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **361**, 2009–2021.

Pomeroy, L.R. & Wiebe, W.J. (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*, **23**, 187–204.

R Development Core Team (2005) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G.A. & Baath, E. (2009) Temperature adaptation of soil bacterial communities along an antarctic climate gradient: predicting response to climate warming. *Global Change Biology*, **15**, 2615–2625.

Rivkin, R.B., Anderson, M.R. & Lajzerowicz, C. (1996) Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. *Aquatic Microbial Ecology*, **10**, 243–254.

Shade, A., Kent, A.D., Jones, S.E., Newton, R.J., Triplett, E.W. & McMahon, K.D. (2007) Interannual dynamics and phenology of bacterial communities in a eutrophic lake. *Limnology and Oceanography*, **52**, 487–494.

Silvius, J.R. (1982) Thermotropic phase transition of pure lipids in model membranes and their modification by membrane proteins. *Lipid–Protein Interactions* (eds P.C. Jost & O.H. Griffith), pp. 239–289, John-Wiley & Sons, New York.

Simon, M., Glockner, F.O. & Amann, R. (1999) Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. *Aquatic Microbial Ecology*, **18**, 275–284.

Sinensky, M. (1974) Homeoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 522–525.

Tanner, R.S. (2005) Cultivation of Bacteria and Fungi. *Manual of Environmental Microbiology* (eds C. Hurst, R. Crawford, J. Garland, D. Lipson, A. Mills & L. Stetzenbach), pp. 62–70, Hoboken, NJ, USA.

van de Vossenberg, J.L.C.M., Driessen, A.J.M., da Costa, M.S. & Konings, W.N. (1999) Homeostasis of the membrane proton permeability in Bacillus subtilis grown at different temperatures. *Biochimica et Biophysica Acta*, **1419**, 97–104.

White, D. (2000) *The Physiology and Biochemistry of Prokaryotes*. Oxford University Press, New York.

Zwart, G., Crump, B.C., Kamst-van Agterveld, M.P., Hagen, F. & Han, S.K. (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquatic Microbial Ecology*, **28**, 141–155.

Received 27 October 2009; accepted 23 February 2010
Handling Editor: Michael Angilletta