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Appl. Environ. Microbiol. **2013, 79(4):1353. DOI: 10.1128/AEM.02753-12.**
Published Ahead of Print 21 December 2012.

AEM
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Ecological Divergence of a Novel Group of *Chloroflexus* Strains along a Geothermal Gradient

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Environmental gradients are expected to promote the diversification and coexistence of ecological specialists adapted to local conditions. Consistent with this view, genera of phototrophic microorganisms in alkaline geothermal systems generally appear to consist of anciently divergent populations which have specialized on different temperature habitats. At White Creek (Lower Geyser Basin, Yellowstone National Park), however, a novel, 16S rRNA-defined lineage of the filamentous anoxygenic phototroph *Chloroflexus* (OTU 10, phylum *Chloroflexi*) occupies a much wider thermal niche than other 16S rRNA-defined groups of phototrophic bacteria. This suggests that *Chloroflexus* OTU 10 is either an ecological generalist or, alternatively, a group of cryptic thermal specialists which have recently diverged. To distinguish between these alternatives, we first isolated laboratory strains of *Chloroflexus* OTU 10 from along the White Creek temperature gradient. These strains are identical for partial gene sequences encoding the 16S rRNA and malonyl coenzyme A (CoA) reductase. However, strains isolated from upstream and downstream samples could be distinguished based on sequence variation at *pcs*, which encodes the propionyl-CoA synthase of the 3-hydroxypropionate pathway of carbon fixation used by the genus *Chloroflexus*. We next demonstrated that strains have diverged in temperature range for growth. Specifically, we obtained evidence for a positive correlation between thermal niche breadth and temperature optimum, with strains isolated from lower temperatures exhibiting greater thermal specialization than the most thermotolerant strain. The study has implications for our understanding of both the process of niche diversification of microorganisms and how diversity is organized in these hot spring communities.

Understanding the factors that contribute to the origins and maintenance of ecological variation is a central goal of the investigation of microbial diversity. Spatially structured environments including physical and chemical gradients are predicted both to enhance diversification rates (1) and to maintain greater diversity through the coexistence of multiple ecological specialists that are locally adapted to different niches (2). The process of ecological specialization entails fitness costs, or trade-offs, whereby a trait that is beneficial in one environment is deleterious in one or more alternative environments. Most evolutionary theory assumes the existence of such trade-offs (3, 4), and evidence for trade-offs is often, but not always, found during the adaptation of experimentally evolved laboratory microorganisms (2, 5, 6).

The contribution of trade-offs to niche diversification in natural communities of microorganisms is less clear, but the environmental gradients of Yellowstone National Park alkaline hot springs are excellent systems for investigating this issue. In particular, trade-offs in thermotolerance appear to be important for structuring diversity in populations of phototrophs in these systems. For example, laboratory strains of the *Synechococcus* A/B group of cyanobacteria isolated from different temperatures from both Yellowstone and Oregon hot springs are ecological specialists with divergent temperature ranges for growth (7, 8), and these genetically distinct lineages are restricted *in situ* to different regions along hot spring thermal gradients (9, 10). Thermal niche specialization has also appeared to have occurred in a group of phototrophic *Acidobacteria* ("*Candidatus* Chloracidobacterium thermophilum"): in White Creek (Lower Geyser Basin, Yellowstone National Park), four different 16S rRNA-defined *Chloracidobacterium* lineages each occupy a relatively narrow (~5 to 15°C) realized thermal niche *in situ* and peak in abundance at different locations along the gradient (10).

These ecologically divergent phototrophic clades often exhibit substantial sequence variation in the highly conserved 16S rRNA gene, suggesting that divergence of these groups is ancient. For example, strains of the *Synechococcus* A/B group characteristic of these systems can vary in 16S rRNA gene sequence by over 5% (7). Given the estimated 50 to 100 million years required for the 16S rRNA gene to diverge by 1% (11), this suggests that the clade began to diverge more than 100 million years ago.

In White Creek, for which the distribution of taxa along its thermal gradient has been extensively investigated (10), members of the genus *Chloroflexus* (phylum *Chloroflexi*) represent an apparent exception to this pattern of ancient specialization. This metabolically versatile group of anoxygenic phototrophs can account for as much as 50% of environmental sequences retrieved from these communities (10). At White Creek, lineages of *Chloroflexus* distinguished by unique sequence tags for the V3 region of the 16S rRNA gene were present in high abundance across a much broader range of temperatures than was observed for other phototrophic groups (10); in particular, one lineage (*Chloroflexus* OTU 10) was the most abundant *Chloroflexi* sequence tag in the full data set (9.0% of the total sequences recovered) and was a major community member (~5 to 25% of the sequences recovered) over a region of the White Creek gradient spanning 39 to 64°C. Its broad distribution raises the question of whether it represents a single generalist or, rather, a group of recently diverged,

Received 6 September 2012 **Accepted** 11 December 2012
Published ahead of print 21 December 2012
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doi:10.1128/AEM.02753-12

cryptic specialists that are indistinguishable by sequencing of the V3 region of the 16S rRNA gene.

Here, we combined phylogenetic analyses of 16S rRNA, malonyl coenzyme A (CoA) reductase, and propionyl-CoA synthase gene sequence data with physiological approaches to address whether cultivated strains of *Chloroflexus* OTU 10 isolated from samples collected along the White Creek gradient have genetically and phenotypically diverged. These are the first cultured members of this group of *Chloroflexi*, which is genetically distinct from the previously cultured members of the genus, including laboratory model strains *Chloroflexus aurantiacus* J-10-fl (12) and *Chloroflexus aggregans* strain DSM9486 (13). Together, the results reveal extensive ecological variation in thermotolerance among closely related White Creek population members of this novel group of *Chloroflexi*.

MATERIALS AND METHODS

Laboratory strain isolation. Microbial mat samples were collected from White Creek (WC) sites WC3 (Universal Transverse Mercator [UTM] coordinates, 516002E 4931137N; approximate temperature, 47°), WC5 (UTM, 516362E 4930907N; approximate temperature, 54°), and WC7 (UTM, 516471E 4930848N; approximate temperature, 61°C) in May/July 2009 and May 2010. Environmental samples were spread on plates of medium D (14) with additions of 0.00082 g/liter anhydrous sodium acetate, 0.0027 g/liter sodium succinate dibasic hexahydrate, 0.0011 g/liter sodium butyrate, 1.87 μg/liter sodium lactate, and 0.00053 g/liter ammonium chloride at pH 8.2. Phytagel (0.7%, wt/vol) was used as the gelling agent, as this has been a successful strategy for improving the cultivation of novel microorganisms from soil (15). Spread plates of the samples collected from WC3 were incubated at 50°C, and spread plates of samples collected from White Creek sites 5 and 7 were incubated at 55°C. Plates were examined under a dissecting microscope several times per week. After 1 to 2 weeks of incubation, isolated colonies with filamentous *Chloroflexus* morphology were transferred to fresh plates. These *Chloroflexi* are capable of gliding motility and will glide away from nonmotile cells with which they are in coculture (16). Forceps were subsequently used to transfer the "leading edge" of the culture to a fresh plate, and this process was repeated until an axenic culture was obtained. A culture was considered axenic if no growth was observed on nutrient agar indicator plates, no contaminating cells were observed by light microscopy, and no sequence heterogeneity was observed for directly sequenced 16S rRNA gene fragments amplified with universal primers by PCR (see below). Strains were maintained in liquid D medium (pH 8.2) with additions of 0.8 g/liter glycylglycine, 2.0 g/liter yeast extract, and 0.2 g/liter ammonium chloride. All cultures were maintained under constant irradiance of 150 μmol photons m^{-2} s^{-1} of tungsten radiation.

DNA isolation, amplification, and sequence analysis. Genomic DNA was isolated from each strain by the method of Pitcher et al. (17). A fragment of the 16S rRNA gene was amplified for each strain using primers 23f and 1492r (18) under the following cycling conditions: 1 min initial denaturing at 94°C; 40 cycles of 1 min at 94°C, 1 min at 54°C, and 1.5 min at 72°C; and a final extension of 10 min at 72°C. Amplified products were directly sequenced with primer 23f at the University of Washington High-Throughput Genomics Unit, Seattle, WA.

Chloroflexi 16S rRNA gene sequences from the metagenomes of White Creek, Chocolate Pots, Fairy Spring, and Bath Lake Vista Annex were obtained from the DOE-JGI Yellowstone Metagenome Project (available at http://img.jgi.doe.gov/cgi-bin/m/main.cgi) (C. G. Klatt, W. P. Inskeep, M. Herrgard, Z. J. Jay, D. B. Rusch, S. G. Tringe, M. N. Parenteau, D. M. Ward, S. M. Boomer, D. A. Bryant, and S. R. Miller, unpublished data). 16S rRNA gene sequence data for *C. aurantiacus* strain J-10-fl, *Chloroflexus* sp. 396-1, and *C. aggregans* strains DSM 9486 and MD-66 were obtained from the NCBI database. These sequences were aligned with 16S rRNA gene sequences from White Creek strains using ClustalW (19). Ambiguous base calls were excluded from the analysis. *Roseiflexus* sequences from the Yellowstone Metagenome Project were also included in the alignment as an outgroup.

Maximum likelihood trees were generated by RaxML version 7.0.3 (20) according to a GTR+G+I model of sequence evolution selected by Modeltest version 3.7 (21). Starting trees were generated randomly, and 100 bootstrap replicates were performed using the rapid bootstrapping algorithm. MrBayes version 3.1.2 (22) was used to generate phylogenies by Bayesian inference. As with the likelihood analysis, the GTR+G+I model was used. Two replicates were run simultaneously for 10,000,000 generations until convergence was attained, as assessed by an average standard deviation of split frequencies below 0.01. To attain convergence between individual analyses, chain swapping was improved by adjusting the heating parameter from the program default of 0.2 to 0.025. The chains were sampled every 100 generations, and the first 20% of sampled trees was discarded as burn-in.

Partial sequences of the malonyl-CoA reductase (*mcr*) and propionyl-CoA synthase (*pcs*) genes were amplified with primers designed using sequences recovered from White Creek and Bath Lake Vista Annex by the Yellowstone Metagenome Project. *mcr* was amplified using forward primer 5′CATCTTTCCCGGCCCGATTG3′ and reverse primer 5′CACA GGCAAATTCTAACCCTTC3′. *pcs* was amplified from each strain using forward primer 5′AGAAGCGTAYACCGATCARG3′ and reverse primer 5′CACCRACCACAATACAATTACC3′. Both sets of primers were designed from the Yellowstone National Park metagenome sequences. Both gene fragments were amplified under the following cycling conditions: 1 min initial denaturing at 94°C; 40 cycles of 1 min at 94°C, 1 min at 54°C, and 2 min at 72°C; and a final extension of 10 min at 72°C. Genes were sequenced using the forward primers at either the University of Washington High-Throughput Genomics Unit or the Murdock Sequencing Facility, The University of Montana, Missoula, MT. Sequences of both genes were aligned with CLUSTALW (19). A sequence of each gene from the Bath Lake Vista Annex metagenome (Klatt et al., unpublished) was also included in the alignments. Identical sequence haplotypes were identified with DnaSP (23). A minimum spanning tree was inferred in Arlequin (24) and manually adjusted to produce a genealogical network for which the distance between any two sequences was identical to the number of observed nucleotide differences between them.

Determination of strain growth temperature ranges. For growth rate experiments to assay temperature performance, strains were grown in liquid D medium (pH 8.2) with additions of 0.8 g/liter glycylglycine, 2.0 g/liter yeast extract, and 0.2 g/liter ammonium chloride. Test tubes were inoculated with enough stationary-phase culture (generally 2 ml) to obtain a starting optical density at 660 nm (OD_{660}) of 0.01 to 0.02. ODs of 2-ml subsamples were determined by spectrophotometry at 660 nm every 24 h until the cultures reached stationary phase (3 to 5 days). Growth rates were measured at 41°C, 45°C, 50°C, 55°C, 58°C, 60°C, 65°C, and 67°C (67°C for strain WC7-3 only) at an irradiance of 150 μmol photons m^{-2} s^{-1} of tungsten radiation. Triplicates of each strain were performed for each temperature treatment. The generation time during exponential growth was estimated by $\log 2/b$, where b is the slope of the logarithmically transformed optical density of a culture at 660 nm regressed on time.

Nucleotide sequence accession numbers. New sequences generated in this study were deposited in GenBank under accession numbers KC154234 to KC154266.

RESULTS

Laboratory strain isolation. Environmental samples were collected from White Creek sites WC3, WC5, and WC7 between May 2009 and May 2010. These sites are oriented along the upstream-downstream axis of the channel, with average temperatures of approximately 47°C, 54°C, and 61°C, respectively (10, 25). We obtained 11 axenic *Chloroflexus* strains from these samples by isolation of colonies from spread plates followed by subsequent plate transfers (Table 1). All strains grew on phytagel plates containing

TABLE 1 Summary of *Chloroflexus* strains isolated with their field collection sites and dates

Strain	Collection site (temp)	Collection date
WC3-1	White Creek 3 (47°C)	May 2009
WC3-2	White Creek 3 (47°C)	May 2009
WC3-3	White Creek 3 (47°C)	July 2009
WC3-4	White Creek 3 (47°C)	July 2009
WC3-5	White Creek 3 (47°C)	July 2009
WC5-1	White Creek 5 (54°C)	May 2010
WC5-2	White Creek 5 (54°C)	May 2010
WC5-4	White Creek 5 (54°C)	May 2010
WC7-1	White Creek 7 (61°C)	May 2010
WC7-2	White Creek 7 (61°C)	May 2010
WC7-3	White Creek 7 (61°C)	May 2010

1% strength medium D supplemented with ammonium and organic acids, but only six were capable of growth in liquid medium. Light microscopy was used to confirm that strains had characteristic *Chloroflexus* morphology, and absorption spectra likewise indicated the presence of bacteriochlorophyll *c* (not shown).

Phylogenetic analyses. All strains were identical to each other for an alignment of 680 nucleotides of the 16S rRNA gene as well as identical to the *Chloroflexus* OTU 10 sequence tag previously recovered from White Creek and Rabbit Creek (10). Phylogenies were reconstructed to determine the evolutionary relationships of these strains to cultured representatives of the genus and to *Chloroflexus* sequences recovered from Yellowstone hot spring metagenomes (Klatt et al., unpublished; http://img.jgi.doe.gov/cgi-bin/m/main.cgi). Bayesian inference and maximum likelihood produced similar results (Fig. 1). The White Creek strains were most similar (99.5 to 100% sequence identity) to sequences that are widespread in other Yellowstone hot springs, particularly Bath Lake Vista Annex (Mammoth Hot Springs), yet are distinct from previously cultured *Chloroflexus* strains (Fig. 1). For example, sequence identity was ~96% to *Chloroflexus* sp. 396-1 and ~94% to *C. aurantiacus* strain J-10-fl.

To determine if the White Creek strains could be distinguished genetically, fragments of the malonyl-CoA reductase (*mcr*) and propionyl-CoA synthase (*pcs*) genes of the 3-hydroxypropionate carbon fixation pathway were sequenced. All White Creek strain sequences were identical for a partial sequence (761 nucleotides) of the *mcr* gene and differed by less than 1% (1/151 nucleotides in the region of overlap) from a sequence recovered in the Bath Lake Vista Annex metagenome. The *mcr* sequence exhibited 75.6% sequence identity to the sequence of *C. aurantiacus* strain J-10-fl. White Creek strains could be distinguished by a partial (657-nucleotide) *pcs* gene sequence, however. Three different *pcs* alleles were observed among the 11 White Creek strains. Each was distinct from the allele detected in the Bath Lake Vista Annex metagenome (Fig. 2A), and average sequence identity with the sequence of *C. aurantiacus* strain J-10-fl was approximately 80%. All WC3 and WC5 strains shared the same allele, two of the three WC7 strains (WC7-1, WC7-2) had a second allele, and strain WC7-3 had a unique sequence (Fig. 2A).

Gene sequences sampled from populations often violate the assumptions of traditional phylogenetics that bifurcating descendants are related by an extinct ancestor and that there has been no recombination between sequences (26). Therefore, to infer the relationships among the *pcs* alleles, a minimum-spanning genealogical network which relaxes these assumptions was reconstructed for the White Creek and Bath Lake Vista alleles (Fig. 2B). This network is characterized by a central loop, which indicates that the evolutionary history of *pcs* has been impacted by either recombination or recurrent mutation. Since all nucleotide sites in the *pcs* alignment consisted of at most only two variants, the data conform to the infinite site mutation model (i.e., there has been a maximum of one mutation at each nucleotide position) and thereby strongly implicate recombination rather than multiple mutations at the same nucleotide position. Specifically, the WC7-3 allele appears to be a recombinant of the other two White Creek alleles: comparison of the variable nucleotide positions in the *pcs* sequence clearly show alternating tracts of sequence identity between WC7-3 and WC7-1 and the WC3 and WC5 alleles, respectively (Fig. 2A). This suggests recent or ongoing gene flow among *Chloroflexus* lineages along the White Creek gradient.

Variation in cardinal growth temperatures. To test for ecological diversification among strains, growth rates for the six strains capable of growth in liquid medium were determined at

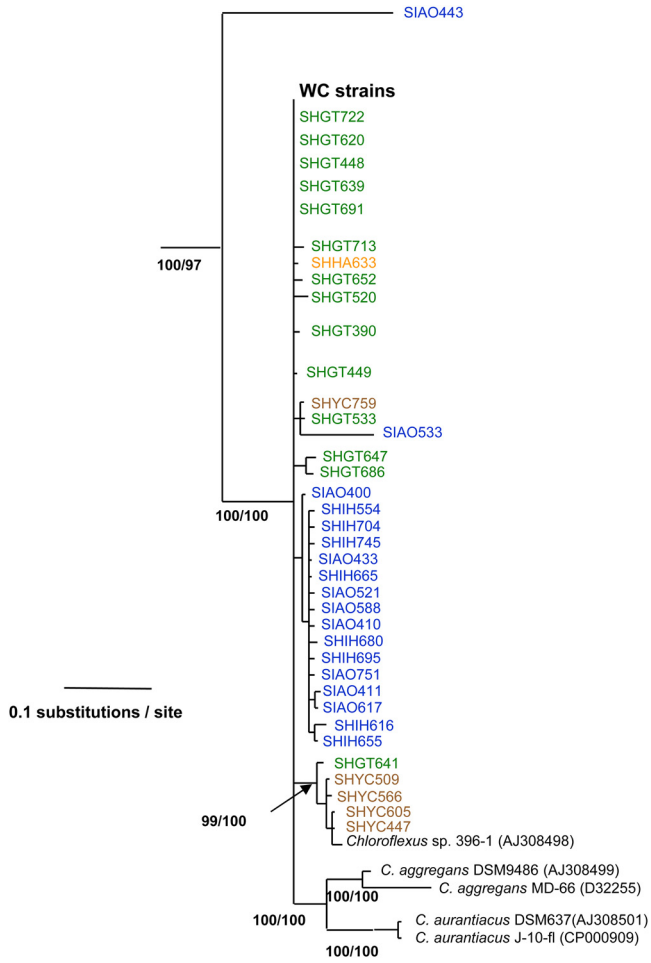

FIG 1 *Chloroflexus* 16S rRNA gene phylogeny including White Creek strains (bold) reconstructed by Bayesian inference and outgroup-rooted with *Roseiflexus* sequences (not shown). Values at nodes represent posterior probabilities from the Bayesian analysis followed by maximum likelihood bootstrap values for 100 bootstrap replicates. Origins of Yellowstone metagenome sequences are distinguished as follows: White Creek, green; Bath Lake Vista Annex, blue; Chocolate Pots, yellow; and Mushroom Spring, brown.

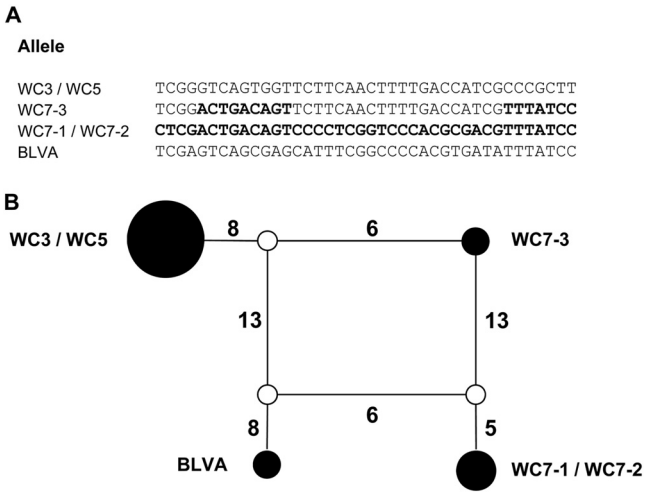

FIG 2 (A) Alignment of the 40 variable nucleotide sites (among 657 aligned nucleotides) in the *pcs* alleles from White Creek (WC) and Bath Lake Vista Annex (BLVA). The WC7-3 allele has a mosaic structure, with alternating tracts of sequence identity with the WC3/WC5 (normal type) and the WC7-1/WC7-2 (boldface type) alleles, respectively. (B) Genealogical network of *pcs* alleles. Branch lengths are in units of number of nucleotide differences between a pair of nodes. The number of nucleotide differences between a pair of alleles is therefore the sum of the branch lengths separating them. Open nodes are alleles which were not observed in the sample.

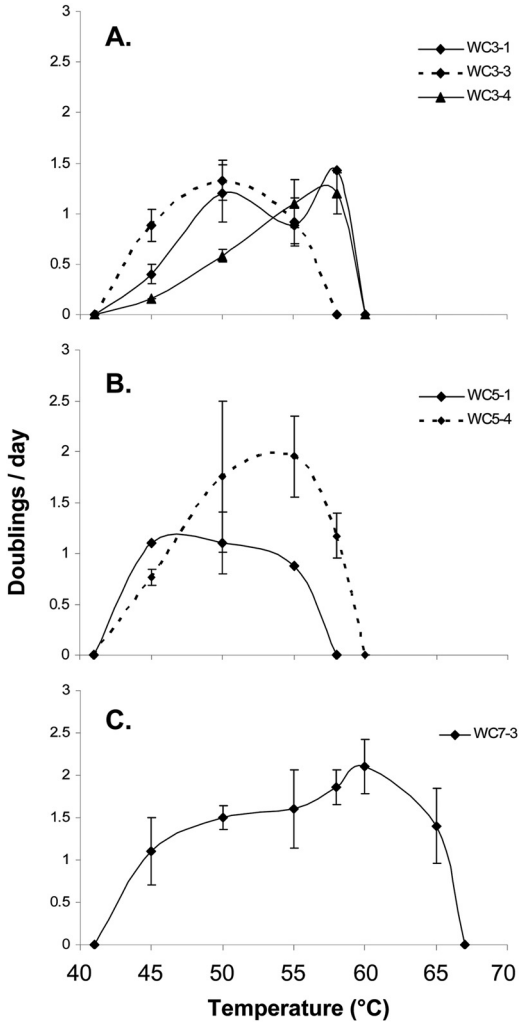

FIG 3 Temperature dependence of exponential growth rates of *Chloroflexus* strains from sites WC3(A), WC5 (B), and WC7 (C), measured by the change in optical density at 660 nm. Error bars represent standard errors for triplicate samples.

seven different temperatures, ranging from 41 to 67°C. Strains isolated from different sites along the White Creek thermal gradient exhibited considerable variation in the temperature dependence of growth (Fig. 3). Strains WC3-3 and WC5-1 exhibited similar thermal reaction norms for growth rate, with an optimum at or below 50°C and no measurable growth at 58°C (Fig. 3A and B). Strains WC3-1, WC3-4, and WC5-4, by contrast, exhibited optimal growth at either 55 or 58°C but did not grow at 60°C (Fig. 3A and B). WC7-3 was the most thermotolerant strain, with maximal growth rate at 60°C and growth at 65°C (Fig. 3C). It also exhibited the broadest temperature range permissive for growth. In fact, there was statistical evidence of borderline significance for general positive correlations both between a strain's optimal temperature and its temperature range breadth ($r = 0.82$, $P = 0.04$; that is, the more thermotolerant a strain is, the broader its thermal niche) and between maximal growth rate and temperature breadth ($r = 0.78$, $P = 0.06$). WC7-3 exhibited the highest maximal growth rate in the data set (2.1 doublings per day at 60°C). This observation reflected a more general trend for optimal temperature to be positively associated with maximal growth rate, though the estimated Pearson correlation coefficient was not significant ($r = 0.53$, $P = 0.28$). Our observation that, at lower temperatures, WC7-3 grew at rates comparable to other strains further suggests that the extension of its thermal niche to a higher maximum cardinal temperature came without a cost in performance at lower temperatures.

DISCUSSION

Recent divergence of White Creek *Chloroflexus*. Like other phototrophs from alkaline geothermal systems, the White Creek *Chloroflexus* OTU 10 lineage has genetically differentiated. In contrast with 16S rRNA-divergent lineages of *Synechococcus* and *Chloracidobacterium* at White Creek, however, a more recent origin of *Chloroflexus* OTU 10 strain diversity is indicated by their 100% sequence identity over a partial 16S rRNA gene fragment. Together with the unique *mcr* and *pcs* alleles that were observed in the White Creek population (Fig. 2), these sequence data favor the interpretation that there has been a comparatively recent adaptive radiation of *Chloroflexus* OTU 10 along the White Creek temperature gradient. Data from additional geographic locations will be required to more conclusively address whether diversification of these *Chloroflexus* strains occurred within White Creek itself.

These differences in time scales of diversification among phototrophic bacteria have implications for our understanding of the history of community structure and dynamics in these systems. An early idea was that a tight producer-consumer relationship between 16S rRNA-defined lineages of *Synechococcus* and *Chloroflexi* (27) drove their ancient coevolution along the thermal gradients of these hot springs (28). Our conclusion that the divergence of the White Creek *Chloroflexus* OTU 10 genotypes occurred much later than the diversification of the three different 16S rRNA-defined *Synechococcus* A/B lineages with which they

collectively cooccur (10) instead suggests that any coevolved ecological interactions between these groups would have to have developed more recently, during or following the diversification of *Chloroflexus*.

Together with more finely resolved investigations of the genetic diversity of these systems, these results also suggest that the association between these two groups is more dynamic than originally proposed. For example, the identity of the *Chloroflexi* partner(s) can vary greatly between hot springs. Our understanding of these interactions comes principally from Octopus Spring and Mushroom Spring, where, over a range of temperatures that is similar to that of White Creek, *Synechococcus* cooccurs primarily with *Roseiflexus* (29) rather than with *Chloroflexus* OTU 10. Moreover, reports of the existence within a single hot spring of multiple, recently diverged *Synechococcus* ecotypes with identical 16S rRNAs (30, 31) further raise the possibility that both the genetic and the ecological diversity of *Synechococcus* may vary among hot spring communities. A potential consequence of the recent and idiosyncratic nature of these *Synechococcus-Chloroflexi* associations is that the outcome of their ecological interactions (e.g., whether they are primarily cooperative or competitive, which metabolites are exchanged) may differ among hot springs. The recently proposed model of cross-feeding of glycolate and fermentation products from *Synechococcus* to *Roseiflexus* for the well-characterized Octopus Spring and Mushroom Spring systems (32) emphasizes positive interactions. At both White Creek and Rabbit Creek, however, pyrosequencing data showed that the relative abundances of the two groups are negatively correlated (10), which suggests that the ecological interactions between these two groups may be primarily competitive. Although cross-feeding and competition are not necessarily mutually exclusive, these latter observations argue for a better understanding of metabolite exchange for *Synechococcus-Chloroflexi* associations in other hot spring communities to investigate the generality of the cross-feeding model.

In several respects, *Chloroflexus* OTU 10 diversity more closely resembles that of the cyanobacterium *Mastigocladus laminosus*, which cooccurs with *Chloroflexus* in White Creek mats between temperatures of 39 and ~55°C (10). White Creek *M. laminosus* strains also exhibit 100% sequence identity at the 16S rRNA locus across a broad range of temperatures but are likewise genetically variable at more rapidly evolving loci. In both populations, ecologically divergent population members are restricted to different locations along the thermal gradient (25). Recombination has played an important role in producing genetic variation in *M. laminosus* (33), and future studies can address whether this is also generally the case for *Chloroflexus*, as we have observed at the *pcs* locus.

***Chloroflexus* OTU 10 ecological variation and the nature of trade-offs in thermotolerance.** Theories of the evolution of environmental tolerance (e.g., see reference 3) commonly assume that the area under organism fitness curves (e.g., the thermal reaction norms for growth rate in Fig. 3) remains constant during diversification. Implicit in this assumption is a "jack-of-all-trades is a master of none" generalist-specialist trade-off, in which an extension in niche breadth necessarily comes at the cost of a reduction in maximal performance. In fact, however, it is possible for generalists to maintain high relative fitness across a wide range of temperatures (that is, a "jack-of-all-temperatures" can be "master of all") (34), and, in some cases, no correlation or even a positive correlation between maximal performance and thermal niche breadth has been reported (35–37). This was the case for our study, for which we observed both a "master-of-all" phenotype for strain WC7-3 and a general positive correlation of borderline statistical significance between maximal performance and temperature breadth. Whether such a "master-of-all" distribution is actually realized *in situ* remains to be investigated and will require greater sampling of *Chloroflexus* OTU 10 diversity than was achieved in the present study.

In addition, the significant positive correlation between optimal temperature and niche breadth is in accord with the related observation that "hotter is broader" for some microorganisms, including G4 phage (37, 38) and *Escherichia coli* propagated in a variable thermal environment (39). As for the relationship discussed above between maximal performance and the breadth of the thermal niche, this result is at odds with a presumed generalist-specialist trade-off, and its mechanistic basis remains unclear.

Finally, our study also bears on the "hotter is better" hypothesis (40), which has been proposed to explain why organisms with relatively high optimal temperatures generally have relatively high maximal performance. The hypothesis is based on the thermodynamic argument that reaction rates are higher at increased temperatures, thereby enhancing performance measures, including growth rate. A majority of case studies representing diverse taxa, including phages, bacteria, plants, and animals, supports the hypothesis (41, 42). Although the correlation between optimal temperature and maximal growth rate was not significant for the *Chloroflexus* OTU 10 data, the sign of the estimated correlation was in the expected direction under this hypothesis. Interestingly, thermophilic *Synechococcus* lineages from these alkaline geothermal systems appear to be an exception: contrary to the predictions of the hotter-is-better hypothesis, *Synechococcus* A/B strains adapted to the highest temperatures had the lowest maximal performance (7, 42). Because the most thermotolerant members of this group define the upper temperature limit for photosynthetic metabolism, it raises the possibility that "hotter is better" is less likely to apply to taxa approaching a fundamental evolutionary constraint.

Concluding remarks. Our discovery of different degrees of ecological specialization among strains raises challenging questions for future investigation regarding the factors which shape the distribution and maintenance of *Chloroflexus* OTU 10 diversity at White Creek. For example, do generalists like strain WC7-3 coexist with less thermotolerant genotypes at downstream sites? The comparable or greater fitness of WC7-3 at lower temperatures compared with that of more specialized strains suggests this possibility (Fig. 3). If, however, more thermotolerant generalist lineages prove to be restricted to more upstream regions of White Creek, then what is responsible for this population structure? At the more downstream WC3 and WC5 sites, the predominant cyanobacterium in the community is *M. laminosus*, whereas it is *Synechococcus* at WC7 (10). Downstream and upstream sites are also distinguished by differences in microbial mat fabric: sites WC3 and WC5 are streamer mats, whereas WC7 is a laminated mat more typical of these environments. Ultimately, consideration of the contribution of ecological interactions with other community members or other physical and chemical aspects of the environment may be required to understand the realized niches of different members of the *Chloroflexus* OTU 10 population at White Creek.

ACKNOWLEDGMENTS

This research was supported by National Science Foundation award number EF-0801999.

We thank Darla Carvey for her technical support and Jim Gannon and Winsor Lowe for comments on earlier versions of the manuscript. We thank David Ward and an anonymous reviewer for their insightful comments and suggestions. We gratefully acknowledge the support of the National Park Service (study number YELL-05482).

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