

Comparative genomics provides evidence for the 3-hydroxypropionate autotrophic pathway in filamentous anoxygenic phototrophic bacteria and in hot spring microbial mats

Christian G. Klatt,^{1*} Donald A. Bryant² and David M. Ward¹

¹Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT, USA.

²Department of Biochemistry and Molecular Biology, The Pennsylvania State University, PA, USA.

Summary

Stable carbon isotope signatures of diagnostic lipid biomarkers have suggested that *Roseiflexus* spp., the dominant filamentous anoxygenic phototrophic bacteria inhabiting microbial mats of alkaline siliceous hot springs, may be capable of fixing bicarbonate via the 3-hydroxypropionate pathway, which has been characterized in their distant relative, *Chloroflexus aurantiacus*. The genomes of three filamentous anoxygenic phototrophic *Chloroflexi* isolates (*Roseiflexus* sp. RS-1, *Roseiflexus castenholzii* and *Chloroflexus aggregans*), but not that of a non-photosynthetic *Chloroflexi* isolate (*Herpetosiphon aurantiacus*), were found to contain open reading frames that show a high degree of sequence similarity to genes encoding enzymes in the *C. aurantiacus* pathway. Metagenomic DNA sequences from the microbial mats of alkaline siliceous hot springs also contain homologues of these genes that are highly similar to genes in both *Roseiflexus* spp. and *Chloroflexus* spp. Thus, *Roseiflexus* spp. appear to have the genetic capacity for carbon dioxide reduction via the 3-hydroxypropionate pathway. This may contribute to heavier carbon isotopic signatures of the cell components of native *Roseiflexus* populations in mats compared with the signatures of cyanobacterial cell components, as a similar isotopic signature would be expected if *Roseiflexus* spp. were participating in photoheterotrophic uptake of cyanobacte-

rial photosynthate produced by the reductive pentose phosphate cycle.

Introduction

The microbial mats that develop in the effluent channels of alkaline siliceous hot springs of Yellowstone National Park are model systems for the study of microbial community ecology, and they are valuable modern analogues to ancient stromatolite formations (Ward *et al.*, 1998; 2006; van der Meer *et al.*, 2000). Based on our molecular and microscopic studies of Octopus and Mushroom Springs, these mat communities are dominated by two groups of phototrophs at 60 and 65°C: unicellular cyanobacteria (*Synechococcus* spp.) and filamentous anoxygenic phototrophs (FAPs) related to *Chloroflexus* and *Roseiflexus* spp. (Nübel *et al.*, 2002). Based on growth in culture (Madigan *et al.*, 1974; Pierson and Castenholz, 1974a) and *in situ* experiments showing light-stimulated uptake of radiolabelled organic substrates (Sandbeck and Ward, 1981; Anderson *et al.*, 1987; Bateson and Ward, 1988), it was previously suggested that FAPs in these mats predominantly use photoheterotrophic metabolism to assimilate low-molecular weight organic compounds cross-fed from the cyanobacteria (Ward *et al.*, 1987). However, stable carbon isotope signatures in lipid biomarkers diagnostic of *Chloroflexus aurantiacus* and *Roseiflexus* spp. (van der Meer *et al.*, 2001; 2002; unpubl. results) were found to be isotopically heavier than those typically observed for cyanobacteria (van der Meer *et al.*, 2000; 2003). This was surprising for a situation involving cross-feeding of metabolites between organisms, in which case similar isotopic signatures would be expected in cell components of both organisms. The heavier isotopic signature of the biomarkers of FAPs in the mat was taken as possible evidence for autotrophic metabolism by a mechanism similar to the autotrophic pathway in *C. aurantiacus* (van der Meer *et al.*, 2000; 2003).

Chloroflexus aurantiacus strain OK-70-fl has been grown photoautotrophically in culture (Madigan and Brock, 1977; Sirevåg and Castenholz, 1979), under which conditions it fixes bicarbonate via the proposed

Received 7 December, 2007; revised 6 March, 2007; accepted 10 March, 2007. *For correspondence. E-mail klatt.christian@gmail.com; Tel. (+1) 406 994 3412; Fax (+1) 406 994 3933.

Table 1. Isolate organisms investigated in this study.

Organism	Isolation source	Reference
<i>Chloroflexus aurantiacus</i> J-10-fl	Sokokura, Hakone area, Japan	Pierson and Castenholz (1974b)
<i>Chloroflexus aggregans</i> MD-66	Okukinu Meotobuchi hot spring, Tochigi Pfc, Japan	Hanada <i>et al.</i> (1995)
<i>Roseiflexus castenholzii</i> HL08	Nakabusa hot spring, Nagano Pfc, Japan	Hanada <i>et al.</i> (2002)
<i>Roseiflexus</i> sp. RS-1	Octopus Spring, WY, USA	Madigan <i>et al.</i> (2005)
<i>Herpetosiphon aurantiacus</i> DSM 785	Birch Lake, MN, USA	Holt and Lewin (1968)

Putative homologues were then used to screen a metagenomic sequence database for Octopus and Mushroom Springs (obtained as part of an NSF Frontiers in Integrative Biological Research project; <http://landresources.montana.edu/FIBR/>; <http://www.tigr.org/tdb/ENVMGX/YNPHS/index.html>; Bhaya *et al.*, unpublished) to determine the *in situ* genetic capacity for the 3-OHP pathway. Once identified, metagenomic homologues of these genes were compared with the sequences of the cultured isolates, particularly *Roseiflexus* sp. strain RS-1, which is a genetically relevant isolate compared with Octopus Spring *Roseiflexus* populations (Madigan *et al.*, 2005).

Results and discussion

Genome annotation evidence of 3-OHP pathway in FAP isolates

Figure 1 shows the bicyclic reactions that have been postulated to comprise the 3-OHP pathway for CO₂ fixation in *C. aurantiacus* and indicates the level to which the steps in the pathway have been experimentally characterized. Homologues of all these genes were found in the genomes of the three phototrophs we examined but not in the genome of *H. aurantiacus*. This inference is based on amino acid identities and similarities derived from BLASTP analyses (Table 2) and from matching to profile hidden Markov models (HMMs) in the PFAM and TIGRFAM databases (Table S1).

Steps 1 and 4 (acyl-CoA carboxylases). Evidence for genes encoding the acyl carboxylase enzymes proposed for steps 1 (acetyl-CoA carboxylase) and 4 (propionyl-CoA carboxylase) is presented together and we refer to homologues of these genes as acetyl-CoA/propionyl-CoA carboxylases, because the substrate specificity for these enzymes is unknown. All five analysed genomes contained open reading frames (ORFs) that correspond to the functional domains of bacterial acetyl-CoA carboxylases (for a review, see Cronan and Waldrop, 2002), which is not surprising given that these genes are also involved in fatty acid metabolism (Strauss and Fuchs, 1993) and are not diagnostic of the 3-OHP pathway. The functional domains of acetyl-CoA carboxylases include the biotin carboxylase

(BC) subunit AccC, the biotin carboxyl carrier protein (BCCP) subunit AccB, and the alpha and beta subunits of the carboxyltransferase components (CT_α and CT_β) AccA and AccD, respectively (Li and Cronan, 1992a,b; Best and Knauf, 1993; Marini *et al.*, 1995; Kimura *et al.*, 2000; Kiatpapan *et al.*, 2001) (Table S1). Additional evidence for the putative *accC* ORFs includes a conserved N-terminal sequence A⁶NRGEIA¹⁴ and a glycine-rich region with the sequence GGGG(K/R)G, consistent with other BC subunits of acyl-CoA carboxylases (Chuakrut *et al.*, 2003). Open reading frames annotated as *accB* shared the biotin binding site motif EAMKM, and the lysine residues predicted to be biotin binding sites have glycine and proline residues flanking them as seen in other BCCP sequences (Samols *et al.*, 1988; Chuakrut *et al.*, 2003).

Roseiflexus sp. RS-1 has two copies of the *accA* and *accD* as determined by HMMs (Table S1), and they are 73 and 62% identical to each other at the amino acid level respectively. The *accA* and *accD* most closely related to the sequences in *C. aurantiacus* are reported in Table 2. The colocalization of an *accC* gene downstream of *accDA* in the *Roseiflexus* sp. RS-1 and *R. castenholzii* genomes provided evidence that these particular genes are likely to be subunits of the same carboxylase. Additionally, these genes are adjacent to genes whose products are predicted to encode enzymes that catalyse steps 2 and 3, suggesting that this carboxylase is involved in the 3-OHP pathway (Fig. 2).

Bacterial propionyl-CoA carboxylases and bifunctional acyl-CoA carboxylases have been characterized in the actinomycetes (Hunaiti and Kolattukudy, 1982; Rodríguez and Gramajo, 1999; Rodríguez *et al.*, 2001; Diacovich *et al.*, 2002; 2004; Gago *et al.*, 2006; Lin *et al.*, 2006; Daniel *et al.*, 2007), and they contain a minimum of two different subunits: the BC and BCCP domains are encoded within the α-subunit (AccA/PccA), and the CT domain lies within the β-subunit (AccB/PccB). Bifunctional acyl-CoA carboxylases have also been described in a proposed alternative 3-OHP pathway in the archaeal family *Sulfolobaceae* in the *Crenarchaeota* (Menendez *et al.*, 1999; Chuakrut *et al.*, 2003; Hügler *et al.*, 2003; Alber *et al.*, 2006; Hallam *et al.*, 2006). Diacovich and colleagues (2004) used the crystal structure of the *Streptomyces coelicolor* PccB and site-directed mutagenesis to determine which residues impart substrate specificity for

Table 2. Per cent amino acid identity and similarity of ORFs coding for known experimentally characterized (bold) and uncharacterized enzymes of the 3-OHP pathway in *C. aurantiacus* to orthologues of *Chloroflexi* isolate genomes.

<i>C. aurantiacus</i> gene	Gene name	Step in pathway	% amino acid identity/similarity				
			<i>C. aggregans</i>	<i>Roseiflexus</i> sp. RS-1	<i>R. castenholzii</i>	<i>H. aurantiacus</i>	
Acetyl/propionyl-CoA carboxylase, carboxyltransferase alpha subunit	<i>accA</i>	1/4	92/97	68/80	69/83	58/76	
Acetyl/propionyl-CoA carboxylase, carboxyltransferase beta subunit	<i>accD</i>	1/4	90/96	67/81	68/82	62/77	
Acetyl/propionyl-CoA carboxylase, biotin carboxyl carrier protein subunit	<i>accB</i>	1/4	88/93	60/70	55/69	54/69	
Acetyl/propionyl-CoA carboxylase, biotin carboxylase subunit	<i>accC</i>	1/4	94/97	75/88	74/88	70/83	
Acetyl/propionyl-CoA carboxylase, carboxyltransferase subunit	CT3	1/4	98/99	86/94	85/92	82/89	
Malonyl-CoA reductase	–	2	88/93	58/70	58/70	ND	
Propionyl-CoA synthase	–	3	90/95	71/82	71/81	ND	
Methylmalonyl-CoA epimerase	–	5	93/98	65/81	65/80	55/71	
Methylmalonyl-CoA mutase, C-terminus	–	6	96/97	84/94	82/91	85/90	
Methylmalonyl-CoA mutase, N-terminus	–	6	96/98	91/96	91/95	ND	
Methylmalonyl-CoA mutase, N-terminus	–	6	79/88	65/78	67/79	ND	
Methylmalonyl-CoA mutase, N-terminus	–	6	95/98	78/85	77/84	72/84	
Succinate dehydrogenase/fumarate reductase, b-cytochrome subunit	–	7	93/98	47/61	46/61	48/64	
Succinate dehydrogenase/fumarate reductase, FeS subunit	–	7	95/97	70/81	70/81	70/84	
Succinate dehydrogenase/fumarate reductase FeS subunit	–	7	97/99	81/88	81/88	76/86	
Fumarate hydratase	–	7	96/97	86/91	85/91	74/84	
Succinyl-CoA : L-malyl-CoA transferase	<i>smtA</i>	8	94/97	91/96	91/96	ND	
Succinyl-CoA : L-malyl-CoA transferase	<i>smtB</i>	8	95/97	88/95	88/96	ND	
L-malyl-CoA/β-methylmalyl-CoA lyase	<i>mclA</i>	9	95/98	94/98	93/97	32/50	
Succinyl-CoA : D-citramalate CoA transferase	<i>sct</i>	12	93/98	78/88	94/96	ND	
D-citramalyl-CoA lyase	<i>ccf</i>	13	82/89	65/79	83/90	ND	

Enzymes catalysing steps 1, 4, 6, 7 and 8 are putatively encoded by multiple genes, and acetyl-CoA/propionyl-CoA carboxylase subunits have multiple paralogs (Table S1). ND, not detected.

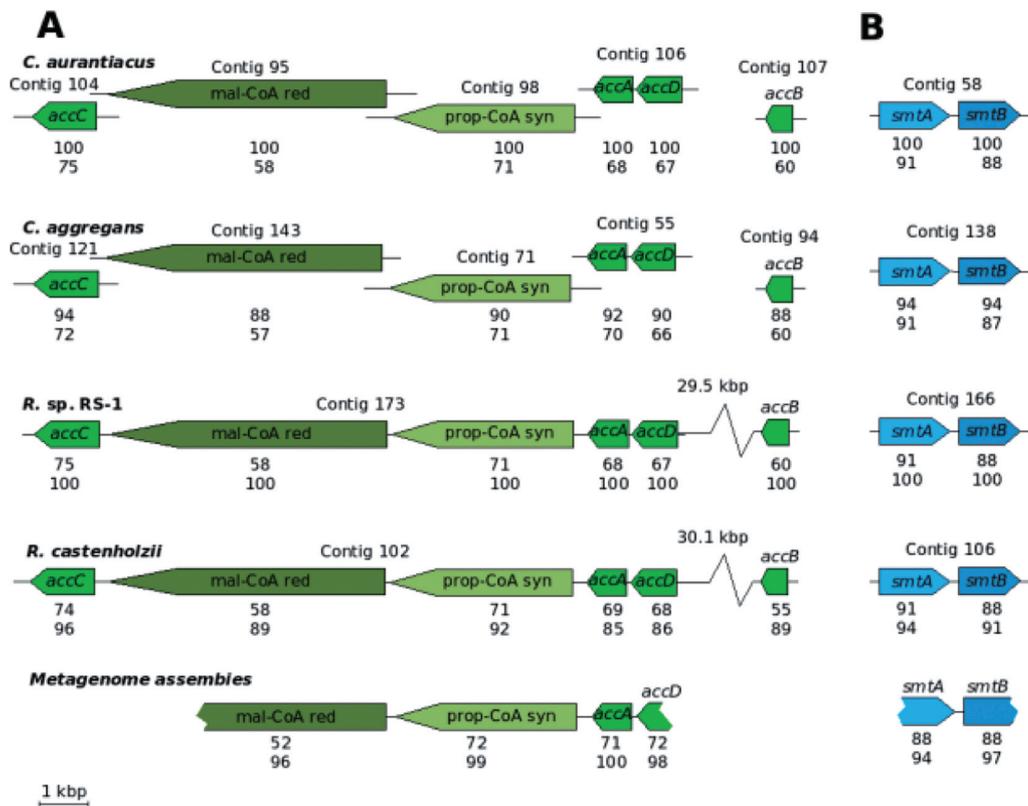


Fig. 2. Locations of genes on isolate genome and metagenome contigs.

A. Acetyl/propionyl-CoA carboxylase (*acc*), malonyl-CoA reductase (*mal-CoA red*), and propionyl-CoA synthase (*prop-CoA syn*).

B. Succinyl-CoA : L-malate CoA transferase (*smtAB*). Zigzag cut-offs represent the ends of fragments of the gene included in the contig. Amino acid identities to *C. aurantiacus* (top) and *Roseiflexus* sp. RS-1 (bottom) are indicated under each gene.

acetyl-CoA and propionyl-CoA. Their findings suggest that bulky hydrophobic residues at position 422 of PccB in *S. coelicolor* (position 473 in Fig. 3A) allow for both acetyl and propionyl-CoA to enter the binding pocket of the active site, whereas an aspartate residue at this position has less affinity for acetyl-CoA. This insight was coupled with a phylogenetic analysis of all FAP ORFs that are predicted to encode a carboxyltransferase domain (Table S1, Fig. 3) to predict carboxyltransferase substrate specificity. From these data, FAP carboxyltransferase ORFs labelled CT3 are predicted to have higher substrate affinity for propionyl-CoA based on the aspartate residue at position 473 (Fig. 3A) and the fact that these sequences cluster with known propionyl-CoA specific carboxyltransferases (Fig. 3B). Filamentous anoxygenic phototroph sequences CT2 and CT4 are predicted to be involved in both acetyl-CoA and propionyl-CoA carboxylase activity, as evidenced by the hydrophobic residue at position 473 and their clustering with bifunctional acyl-CoA carboxylases (Fig. 3B). Open reading frames labelled CT1 are phylogenetically distant from experimentally characterized acyl-CoA carboxylases, and the function of these carboxyltransferases remains unexplored. It should be noted that each analysed

genome has multiple copies of putative BC, BCCP and CT subunits as determined from HMMs (Table S1), and these subunits could combine to form isoenzymes with varying substrate specificities.

Step 2 (malonyl-CoA reductase). Malonyl-CoA reductase catalyses the NADPH-dependent two-step reduction of malonyl-CoA to 3-hydroxypropionate via a malonate semialdehyde intermediate (Hügler *et al.*, 2002). Open reading frames identified as homologues to this gene had statistically significant hits to PFAM models indicating domains conserved in NAD-dependent epimerases and short-chain aldehyde/alcohol dehydrogenases (Table S1), consistent with earlier investigations of the function of this enzyme in *C. aurantiacus* OK-70-fl (Hügler *et al.*, 2002).

Step 3 (propionyl-CoA synthase). The trifunctional enzyme propionyl-CoA synthase activates 3-hydroxypropionate to 3-hydroxypropionyl-CoA, which is then converted to acrylyl-CoA and reduced to propionyl-CoA (Alber and Fuchs, 2002). According to profile HMMs from the PFAM database, this enzyme shares the

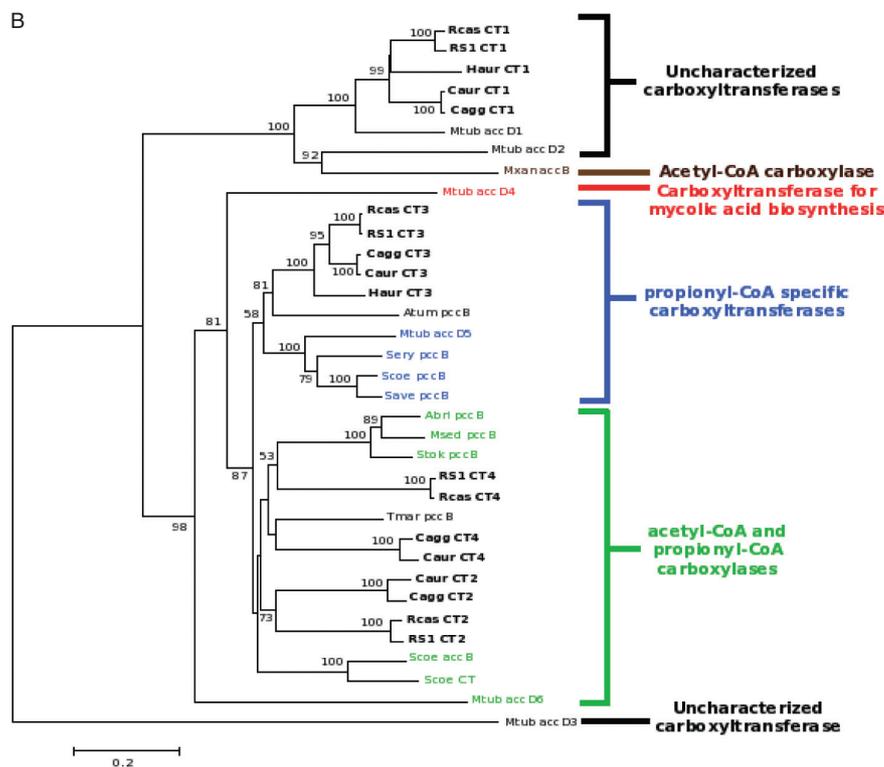
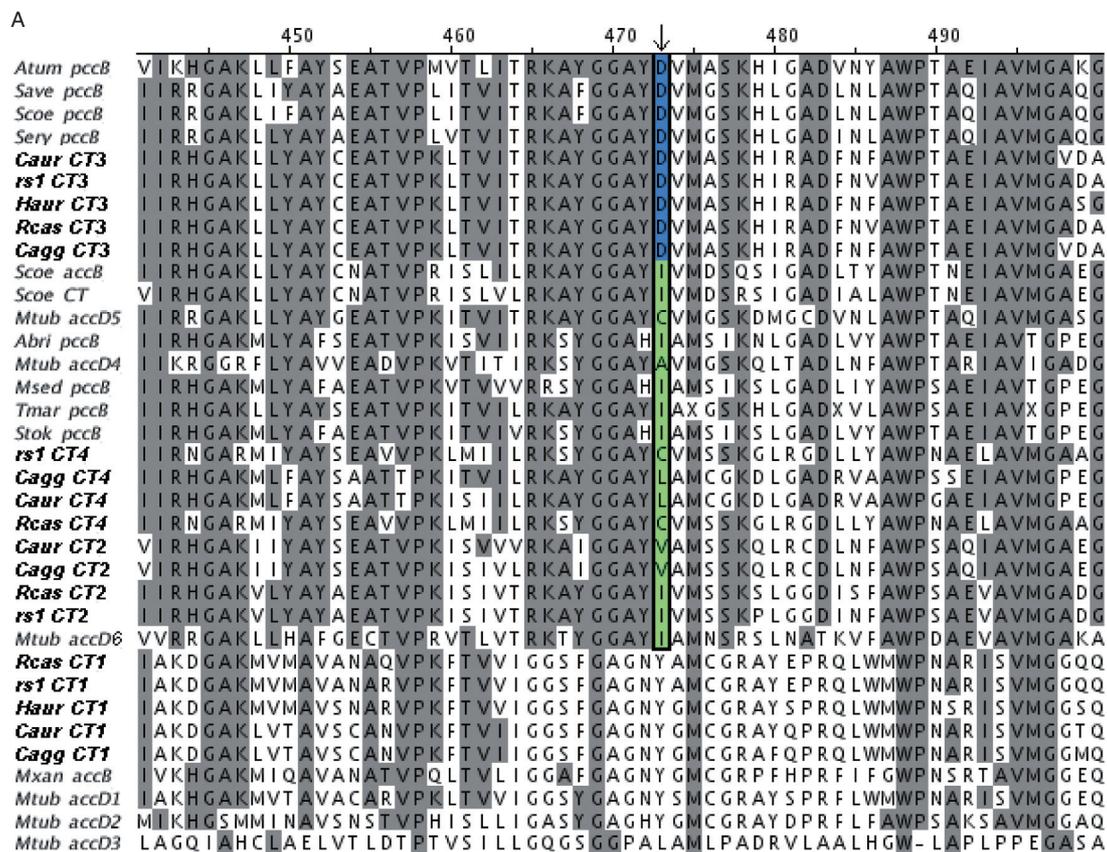


Fig. 3. A. Partial alignment of *Chloroflexi* (bold) and experimentally characterized prokaryotic carboxyltransferases. The marked residue at position 473 imparts substrate specificity in *Streptomyces coelicolor*. Shaded residues indicate $\geq 50\%$ amino acid consensus. Blue aspartate residues show predicted preferential specificity for propionyl-CoA, while green hydrophobic residues indicate predicted specificity for both acetyl-CoA and propionyl-CoA.

B. Phylogenetic analysis of prokaryotic carboxyltransferases. This unrooted neighbour-joining tree shows bootstrap values over 50% (out of 1000 replicates). Horizontal branch lengths are proportional to inferred evolutionary distances, with the scale bar indicating the number of substitutions per site. Names in bold refer to *Chloroflexi* sequences, while coloured names indicate proteins that have been experimentally characterized with respect to substrate specificity for acetyl-CoA or propionyl-CoA. Organisms include the following: Abri, *Acidianus brierleyi*; Atum, *Agrobacterium tumefaciens*; Cagg, *C. aggregans*; Caur, *C. aurantiacus*; Haur, *H. aurantiacus*; Msed, *Metallosphaera sedula*; Mtub, *Mycobacterium tuberculosis*; Mxan, *Myxococcus xanthus*; Rcas, *R. castenholzii*; rs1, *Roseiflexus* sp. RS-1; Sery, *Saccharopolyspora erythraea*; Save, *Streptomyces avermitilis*; Scoe, *S. coelicolor*; Stok, *Sulfolobus tokodaii*; Tmar, *Thermus maritimus*.

conserved domain structure of other enoyl-CoA hydratases and includes an AMP binding site, which is consistent with the findings of Alber and Fuchs (2002) (Table S1). Additionally, a NAD(P)H binding motif of (GXGX₂AX₃A) was found in the sequences from all four phototrophic genomes, with *C. aggregans* sequence exhibiting two such motifs. *Herpetosiphon aurantiacus* does not contain any ORFs that have sequence similarity as statistically significant as our expectation value cut-off to malonyl-CoA reductase or propionyl-CoA synthase (Table 2).

Steps 5–7 (methylmalonyl-CoA epimerase, methylmalonyl-CoA mutase, succinate dehydrogenase and fumarate hydratase). The enzymes in the pathway that convert methylmalonyl-CoA to succinyl-CoA (steps 5 and 6) are also used to oxidize fatty acid chains with an odd number of carbons, while those catalysing the conversion of succinyl-CoA to L-malate (step 7) are also components of the TCA cycle. Evidence of their putative function comes in the form of highly specific (equivalog-level) profile HMMs from the TIGRFAM database. Homologues to genes encoding enzymes catalysing these three enzymatic steps were also found in *H. aurantiacus*.

Step 8 (succinyl-CoA : L-malate-CoA transferase). Two subunits make up the enzyme succinyl-CoA : L-malate-CoA transferase (SmtA and SmtB), which is a Type III CoA transferase (Friedmann *et al.*, 2006a). This family level function was predicted by a PFAM HMM for both SmtA and SmtB (Table S1) in each *Chloroflexi* genome except that of *H. aurantiacus*.

Step 9 (L-malyl-CoA/ β -methylmalyl-CoA lyase). The proposed 3-OHP pathway is bicyclic in that the glyoxylate produced in the first cycle acts as the intermediate that is used in a second cycle to produce pyruvate. L-malyl-CoA/ β -methylmalyl-CoA lyase has been demonstrated to have the dual function of cleaving L-malyl-CoA to acetyl-CoA and glyoxylate (thus completing the first cycle), and then condensing glyoxylate with propionyl-CoA to produce β -methylmalyl-CoA (which begins the second cycle) (Herter *et al.*, 2002). Sequences showing similarity to the L-malyl-CoA/ β -methylmalyl-CoA lyase were also pre-

dicted to have aldolase/citrate lyase activity (Table S1), consistent with the results of Herter and colleagues (2002) (Table S1). A homologue of L-malyl-CoA/ β -methylmalyl-CoA lyase (step 9) in *C. aurantiacus* was also found in *H. aurantiacus*. However, these predicted proteins were not very similar in sequence, and therefore these gene products may not share the same function (Table 2).

Steps 10 and 11. The enzymes proposed to convert β -methylmalyl-CoA via the intermediate mesaconyl-CoA to D-citramalate (steps 10 and 11) have not yet been identified and characterized.

Steps 12 and 13 (succinyl-CoA/D-citramalate CoA transferase and D-citramalyl-CoA lyase). A second Type III CoA transferase has been shown to catalyse the reaction in step 12 in which D-citramalate is converted to D-citramalyl-CoA (Friedmann *et al.*, 2006b). Homologues to this sequence in *C. aurantiacus* are also predicted to have Type III CoA transferase activity as determined from HMMs (Table S1). A D-citramalyl-CoA lyase gene is adjacent to this gene in *C. aurantiacus*, and its function in catalysing step 13 in the pathway was predicted and confirmed by Friedmann and colleagues (2006b). The PFAM model for this sequence is not sufficiently specific to identify the CoA lyase function in the genomes of the four phototrophs.

Similarity in genes and gene order in *Chloroflexus* and *Roseiflexus*

All of the genes encoding enzymes of the 3-OHP pathway in *C. aurantiacus* have greater amino acid identities and similarities to their homologues in *C. aggregans* than to the homologues in the two *Roseiflexus* spp. (Table 2). This is consistent with the greater phylogenetic relatedness between the two *Chloroflexus* species than between *Chloroflexus* and *Roseiflexus* species. The 16S rRNA sequences of *C. aurantiacus* is 92% identical to that of *C. aggregans*, but it is only 83% identical to those of both *Roseiflexus* sp. RS-1 and *R. castenholzii*. The order and direction of ORFs predicted to encode enzymes of the 3-OHP pathway provided additional evidence that these genes are used in this pathway. For instance, Fig. 2

shows contigs in the draft *Roseiflexus* spp. genomes in which ORFs encoding subunits of an acetyl-CoA/propionyl-CoA carboxylase (step 1 or 4) are adjacent to those encoding the unique 3-OHP pathway genes malonyl-CoA reductase (step 2) and propionyl-CoA synthase (step 3). In *Chloroflexus* spp., only *accA* and *accD* are adjacent on the same contig, while the *accC*, malonyl-CoA reductase and propionyl-CoA synthase genes are each found on different contigs and are surrounded by neighbouring genes that do not encode enzymes in the pathway. The observed synteny between isolates of each species, but the absence of synteny between isolates of the two different genera, is also consistent with the greater phylogenetic distance separating *Roseiflexus* strains and *Chloroflexus* strains.

Absence of alternative autotrophic pathways

To determine whether these organisms have the potential to use carbon fixation pathways other than the 3-OHP pathway, TBLASTN was used to query the genome sequences for evidence of other carboxylase genes. As defined by the criteria described in the *Experimental procedures*, none of the five genomes that were analysed in this study appear to contain homologues to (i) ribulose-1,5-bisphosphate carboxylase/oxygenase (Calvin-Benson-Bassham cycle) from *O. trichoides* (GenBank Accession AAZ52657), (ii) carbon monoxide dehydrogenase (Accession P31896) and acetyl-CoA synthase (Accession P27988) from experimentally characterized protein sequences (both in the Wood-Ljungdahl or reductive acetyl-CoA pathway) or (iii) ATP-dependent citrate lyase (Accessions AAM72322 and AAM72321), and 2-oxoglutarate : ferredoxin oxidoreductase from *Chlorobium tepidum* (Accessions AAM71411 and AAM71410) (both in the reductive tricarboxylic acid cycle) (data not shown). Despite the lack of evidence of other autotrophic pathways in these genomes, all *Chloroflexus* and *Roseiflexus* genomes contain an ORF that is homologous to pyruvate : flavodoxin/ferredoxin oxidoreductase, an enzyme that can be used to either decarboxylate pyruvate, or synthesize pyruvate by carboxylation of acetyl-CoA in an anapleurotic pathway (Raymond, 2005). The latter reaction was proposed to operate in an autotrophic reductive cycle of dicarboxylic acids in *C. aurantiacus* strain B-3 (Ugol'kova and Ivanovsky, 2000).

These genomic comparisons have allowed us to identify the potential of three phototrophic *Chloroflexi* to perform the 3-OHP pathway for autotrophy despite the present limitation of not being able to grow these isolates autotrophically in culture. The inability to grow these strains autotrophically could result from the failure to identify a suitable electron donor for autotrophic growth, or it could reflect the possibility that these *Chloroflexi* strains

can only grow mixotrophically by oxidizing organic compounds while at the same time fixing some CO₂ via the 3-OHP pathway. An analogous strategy is used by the aerobic anoxygenic phototroph *Roseobacter denitrificans*, which lacks a definitive autotrophic pathway, yet still demonstrates light-stimulated uptake of CO₂ (Swingley *et al.*, 2007). *Roseiflexus castenholzii* was tested for photoautotrophic growth using Na₂S₂O₃ and Na₂S as electron donors (Hanada *et al.*, 2002) but it is possible that *Roseiflexus* spp. are capable of using H₂ as an electron donor as evidenced by a putative membrane bound Group 1 [Ni-Fe] uptake hydrogenase enzyme in both *R. castenholzii* and *Roseiflexus* sp. RS-1 genomes (Accession numbers: *R. castenholzii*, ZP_01531052 and ZP_01531053; *Roseiflexus* sp. RS-1, ZP_01357085 and ZP_01357084) (Vignais *et al.*, 2001).

Other than the above-mentioned difference in gene organization, this study revealed the potential for a 3-OHP pathway in the three FAPs studied, and this pathway is similar to the proposed pathway in *C. aurantiacus*. A similar comparative approach of genomic and metagenomic data has been applied to find evidence of the 3-OHP pathway in organisms of the *Crenarchaeota* (Hallam *et al.*, 2006), which use an alternative malonyl-CoA reductase enzyme (Alber *et al.*, 2006) and use a modified 3-OHP pathway (Hügler *et al.*, 2003).

Environmental genomic analysis

The 3-OHP pathway homologues identified in both *C. aurantiacus* and *Roseiflexus* sp. RS-1 genomes showed high amino acid sequence identity to the translations of environmental DNA sequences obtained by shotgun cloning and clone-end sequencing from Octopus and Mushroom Spring mat samples collected from sites with average temperatures of 60°C and 65°C (Fig. 4). It is clear that homologues of genes involved in the 3-OHP pathway from both *Chloroflexus* and *Roseiflexus* spp. are present in the mat. For each gene, multiple homologous reads with different sequences were observed. Reads encoding homologues more closely related to *Roseiflexus* sp. RS-1 (126 reads ≥ 90% amino acid identity to *Roseiflexus* sp. RS-1) outnumber those to *C. aurantiacus* (61 reads ≥ 90% amino acid identity for *C. aurantiacus*). Reads encoding homologues that are more closely related to *C. aurantiacus* genes are more abundant in the high temperature (65°C) clone libraries, consistent with previous data showing greater relative abundance of *Roseiflexus* spp. at 60°C, and a greater abundance of *Chloroflexus* spp. at the higher temperature (Nübel *et al.*, 2002). The lower sequence identity of metagenomic homologues (Fig. 4) to *C. aurantiacus* strain J-10-fl protein sequences may be due to the phylogenetic distance separating this Japanese isolate and populations

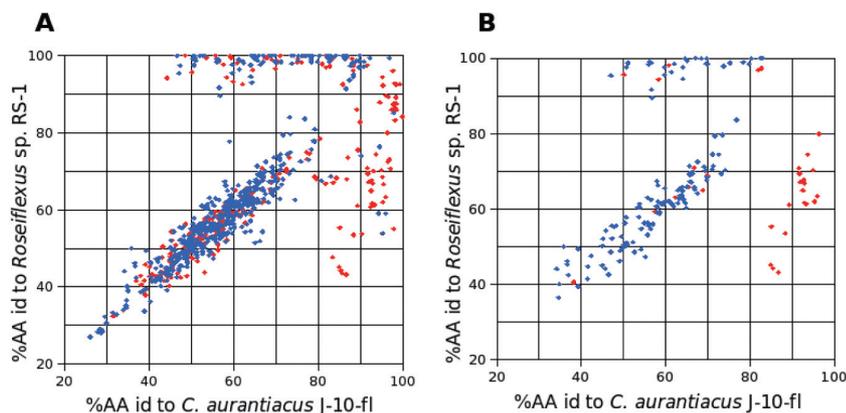


Fig. 4. Per cent amino acid identity of metagenome sequences encoding 3-OHP pathway genes to homologues in the *C. aurantiacus* and *Roseiflexus* sp. RS-1 genomes. Blue and red symbols indicate metagenome reads from low temperature (average 60°C), and high temperature (65°C) sites respectively.

A. All homologues putatively involved in the 3-OHP pathway in *C. aurantiacus* and *Roseiflexus* sp. RS-1 (811 reads in a reciprocal TBLASTN/BLASTX search). B. The subset of 172 reads hitting malonyl-CoA reductase or propionyl-CoA synthase, which catalyse the two unique steps of the pathway.

inhabiting Yellowstone hot springs (Nübel *et al.*, 2002). Metagenome read sequences that are less than 80% identical to either isolate are too phylogenetically distant to infer their function.

The colocalization of 3-OHP pathway genes on a contig assembled from the metagenome provided additional evidence of autotrophic capability in uncultured *Roseiflexus* spp. (Fig. 2). This contig contains four 3-OHP pathway genes, including those encoding two acyl-CoA carboxylase subunits and the diagnostic enzymes malonyl-CoA reductase and propionyl-CoA synthase, and these are arranged in the same order as found in *Roseiflexus* isolate genomes. A BLASTX comparison of translated metagenomic sequences to homologous amino acid sequences in the isolate genomes indicate that the genes on this contig are more closely related to genes of *Roseiflexus* sp. RS-1 than to genes of *C. aurantiacus* (Fig. 2). The *smtAB* (step 8) homologues (Friedmann *et al.*, 2006a) were similarly found to be adjacent on a 1.8-kb contig assembled from the metagenome and showed 94% and 88% amino acid identity to *smtA* homologues and 97% and 88% amino acid identity to the *smtB* homologues of *Roseiflexus* sp. RS-1 and *C. aurantiacus*, respectively (Fig. 2).

Conclusions

The results reported here support the hypothesis that the dominant *Roseiflexus* populations in the microbial mats of alkaline siliceous hot springs have the capacity to fix inorganic carbon via the 3-OHP pathway. These results provide a basis for inferences made previously from other evidence that autotrophy via this pathway is one mechanism that can lead to heavier ^{13}C signatures in *Roseiflexus* spp. biomarkers compared with *Synechococcus* spp. biomarkers. The crucial next steps will be to verify these *in silico* predictions by demonstrating the expression of genes encoding 3-OHP pathway enzymes in the mat. We will use the gene sequences we have reported

here to study the contributions of *Roseiflexus* and *Chloroflexus* populations to the overall inorganic carbon fixation in these mats over a diel time-course. These studies will use enzyme activity measurements combined with quantitative reverse transcription polymerase chain reaction analysis of mRNA transcripts, which is an approach that has been successfully used to measure *in situ* expression of *Synechococcus* genes in these same mats (Steunou *et al.*, 2006).

Experimental procedures

Metagenome library construction and assembly

Core samples (0.5 cm²) of variable depth were taken in the afternoons of 2 October 2003 and 5 November 2004 from 60°C and 65°C cyanobacterial mats in Octopus and Mushroom Springs in Yellowstone National Park, Wyoming, USA. These were sectioned in the field into ~1 mm thick depth intervals using a razor blade and quickly frozen on liquid nitrogen. Two lysis protocols were used: (i) a mechanical bead-beating lysis and (ii) an enzymatic lysis using lysozyme and proteinase K (details can be found in the *Supplementary material*). The mechanical bead-beating procedure was insufficient to lyse the cells completely, resulting in an over-representation of DNA from organisms that are easily lysed. Thus, clone libraries constructed from mechanically lysed cells are assumed to be atypical of the mat environment in terms of the relative abundance of organisms sampled. Two sets of metagenomic clone libraries were constructed from the DNA extracted from the enzymatically and mechanically lysed cells. The first set was from the top 1 mm of the cores sampled, resulting in the ~167 500 kb of DNA sequence reported in D. Bhaya and colleagues (unpublished). The second data set came from deeper layers of Mushroom Spring cores, and added an additional 18 700 kb of sequence. Table S2 in the *Supplementary material* details the metagenomic library sources and layers analysed in this study.

The Celera assembler was used on the subset of metagenome reads from Octopus Spring resulting in 5757 contigs with an average size of 2.4 kb. These sequences are available on a website: <http://www.tigr.org/tdb/ENVMGX/YNPHS/index.html>

BLAST comparisons

The isolate genome sequences were screened for homologues to genes involved in experimentally characterized steps of the 3-OHP pathway in *C. aurantiacus* OK-70-fl (GenBank Accession numbers AAS20429, AAL47820, ABF14399 and ABF14400) using TBLASTN (Gish, 2007) against the genome contigs. Open reading frames exhibiting alignments at least 100 amino acids long and having expectation values more significant than $1 \times e^{-15}$ were then reciprocally used in a BLASTP search against the NCBI nr database. The same method was used to query the genomes for the presence of carboxylases involved in alternative autotrophic pathways. For steps in the 3-OHP pathway that have not yet been characterized, ORFs were selected for putative gene products that corresponding to predicted functions in the pathway via profile hidden Markov models (see below). All identified ORFs were queried against the *C. aurantiacus* J-10-fl genome in a reciprocal BLASTP analysis with the parameters `hitdist = 40`, `wordmask = seg`, and `postsw` set to obtain the values listed in Table 2.

Homologous sequences among metagenomic reads were found using protein sequences from the genomes as a query in a TBLASTN search against the nucleotide metagenome database. Reads produced alignments of less than 100 amino acids long and with expectation values greater than $1 \times e^{-15}$ and reads that resulted from the biased mechanical lysis protocol were not analysed. A reciprocal BLASTX search of nucleotide metagenome reads against the translated genome peptide databases was used to verify that each read aligned to the original query sequence as the best scoring match.

Hidden Markov Model analysis

Support for the annotations of the ORFs predicted to encode proteins of the 3-OHP pathway came from the program HMMER 2.3.2 (Eddy, 1998) (<http://selab.janelia.org/>), which determines statistically significant matches to profile hidden Markov models (HMMs). This program is used to screen ORFs for conserved domains, which provide functional evidence for gene annotation. Models that scored above the trusted cut-offs in the curated TIGRFAM and PFAM profile HMM databases are reported with their corresponding expectation values in Table S1.

Phylogenetic analysis

Experimentally characterized carboxyltransferase sequences were obtained from GenBank. An alignment was constructed using Clustalx and was manually edited in MEGA3.1 (Kumar *et al.*, 2004). The neighbour-joining tree was constructed using MEGA3.1 with 1000 bootstrap replicates.

Acknowledgements

This work was funded by the NASA Exobiology Program (NAG5-8824), the Montana State University Thermal Biology Institute (NASA NAG5-8807) and an NSF Frontiers in Integrative Biological Research award (EF-0328698) to D.M.W. This work was also funded by NSF Grant MCB-0523100 to

D.A.B. We thank J.F. Heidelberg (University of Southern California) for creating the metagenomic sequence assemblies analysed in this work, S. Hanada (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan), M.T. Madigan (Southern Illinois University) and B.K. Pierson (University of Puget Sound) for providing *Chloroflexus aggregans*, *Chlorothrix halophila*, *Roseiflexus* sp. RS-1, and *R. castenholzii* cultures used to obtain genomes, M.M. Bateson (Montana State University) for providing DNA extracts, M.A. McClure (Montana State University) for assistance with the phylogenetic work, and J.W. Peters (Montana State University) for helpful suggestions. We thank R.E. Blankenship (Washington University), B.K. Pierson, and P. Richardson at the Department of Energy Joint Genome Institute (http://genome.jgi-psf.org/mic_home.html) for producing and giving us permission to use the genome sequence of *Chloroflexus aurantiacus* J-10-fl.

References

- Alber, B.E., and Fuchs, G. (2002) Propionyl-coenzyme A synthase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO₂ fixation. *J Biol Chem* **277**: 12137–12143.
- Alber, B., Olinger, M., Rieder, A., Kockelkorn, D., Jobst, B., Hügler, M., and Fuchs, G. (2006) Malonyl-coenzyme A reductase in the modified 3-hydroxypropionate cycle for autotrophic carbon fixation in archaeal *Metallosphaera* and *Sulfolobus* spp. *J Bacteriol* **188**: 8551–8559.
- Anderson, K.L., Tayne, T.A., and Ward, D.M. (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. *Appl Environ Microbiol* **53**: 2343–2352.
- Bateson, M.M., and Ward, D.M. (1988) Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. *Appl Environ Microbiol* **54**: 1738–1743.
- Berg, I.A., Keppen, O.I., Krasil'nikova, E.N., Ugol'kova, N.V., and Ivanovsky, R.N. (2005) Carbon metabolism of filamentous anoxygenic phototrophic bacteria of the family *Oscillochloridaceae*. *Mikrobiologiya (English translation)* **74**: 258–264.
- Best, E.A., and Knauf, V.C. (1993) Organization and nucleotide sequences of the genes encoding the biotin carboxyl carrier protein and biotin carboxylase protein of *Pseudomonas aeruginosa* acetyl coenzyme A carboxylase. *J Bacteriol* **175**: 6881–6889.
- Chuakrut, S., Arai, H., Ishii, M., and Igarashi, Y. (2003) Characterization of a bifunctional archaeal acyl coenzyme A carboxylase. *J Bacteriol* **185**: 938–947.
- Cronan, J.E., and Waldrop, G.L. (2002) Multi-subunit acetyl-CoA carboxylases. *Prog Lipid Res* **41**: 407–435.
- Daniel, J., Oh, T.-J., Lee, C.-M., and Kolattukudy, P.E. (2007) AccD6, a member of the Fas II locus, is a functional carboxyltransferase subunit of the acyl-coenzyme A carboxylase in *Mycobacterium tuberculosis*. *J Bacteriol* **189**: 911–917.
- Diakovich, L., Peirú, S., Kurth, D., Rodríguez, E., Podestá, F., Khosla, C., and Gramajo, H. (2002) Kinetic and structural analysis of a new group of acyl-CoA carboxylases found in *Streptomyces coelicolor* A3(2). *J Biol Chem* **277**: 31228–31236.

- Diacovich, L., Mitchell, D.L., Pham, H., Gago, G., Melgar, M.M., Khosla, C., *et al.* (2004) Crystal structure of the β -subunit of acyl-CoA carboxylase: structure based engineering of substrate specificity. *Biochemistry* **43**: 14027–14036.
- Eddy, S.R. (1998) Profile hidden Markov models. *Bioinformatics* **14**: 755–763.
- Friedmann, S., Steindorf, A., Alber, B.E., and Fuchs, G. (2006a) Properties of succinyl-coenzyme A:L-malate coenzyme A transferase and its role in the autotrophic 3-hydroxypropionate cycle of *Chloroflexus aurantiacus*. *J Bacteriol* **188**: 2646–2655.
- Friedmann, S., Alber, B.E., and Fuchs, G. (2006b) Properties of succinyl-coenzyme A:D-citramalate coenzyme A transferase and its role in the autotrophic 3-hydroxypropionate cycle of *Chloroflexus aurantiacus*. *J Bacteriol* **188**: 6460–6468.
- Gago, G., Kurth, D., Diacovich, L., Tsai, S.-C., and Gramajo, H. (2006) Biochemical and structural characterization of an essential acyl coenzyme A carboxylase from *Mycobacterium tuberculosis*. *J Bacteriol* **188**: 477–486.
- Gish, W. (2007) WU-BLAST [WWW document]. URL <http://blast.wustl.edu>
- Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M., and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PLoS Biol* **4**: e95.
- Hanada, S., Hiraishi, A., Shimada, K., and Matsuura, K. (1995) *Chloroflexus aggregans* sp. nov., a filamentous phototrophic bacterium which forms dense cell aggregates by active gliding movement. *Int J Syst Bacteriol* **45**: 676–681.
- Hanada, S., Takaichi, S., Matsuura, K., and Nakamura, K. (2002) *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. *Int J Syst Bacteriol* **52**: 187–193.
- Herter, S., Busch, A., and Fuchs, G. (2002) L-Malyl-coenzyme A lyase/ β -methylmalyl-coenzyme A lyase from *Chloroflexus aurantiacus*, a bifunctional enzyme involved in autotrophic CO₂ fixation. *J Bacteriol* **184**: 5999–6006.
- Holo, H., and Sirevåg, R. (1986) Autotrophic growth and CO₂ fixation of *Chloroflexus aurantiacus*. *Arch Microbiol* **145**: 173–180.
- Holt, J.G., and Lewin, R.A. (1968) *Herpetosiphon aurantiacus* gen. nov. sp. nov., a new filamentous gliding organism. *J Bacteriol* **95**: 2407–2408.
- Hügler, M., Menendez, C., Schägger, H., and Fuchs, G. (2002) Malonyl-coenzyme A reductase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO₂ fixation. *J Bacteriol* **184**: 2404–2410.
- Hügler, M., Krieger, R.S., Jahn, M., and Fuchs, G. (2003) Characterization of acetyl-CoA/propionyl-CoA carboxylase in *Metallosphaera sedula*. *Eur J Biochem* **270**: 736–744.
- Hunaiti, A.R., and Kolattukudy, P.E. (1982) Isolation and characterization of an acyl-coenzyme A carboxylase from an erythromycin-producing *Streptomyces erythreus*. *Arch Biochem Biophys* **216**: 362–371.
- Ivanovsky, R.N., Fal, Y.I., Berg, I.A., Ugol'kova, N.V., Krasil'nikova, E.N., Keppen, O.I., *et al.* (1999) Evidence for the presence of the reductive pentose phosphate cycle in a filamentous anoxygenic photosynthetic bacterium, *Oscillochloris trichoides* strain DG-6. *Microbiology* **145**: 1743–1748.
- Kiatpapan, P., Kobayashi, H., Sakaguchi, M., Ono, H., Yamashita, M., Kaneko, Y., and Murooka, Y. (2001) Molecular characterization of *Lactobacillus plantarum* genes for β -ketoacyl-acyl carrier protein synthase III (*fabH*) and acetyl coenzyme A carboxylase (*accBCDA*), which are essential for fatty acid biosynthesis. *Appl Environ Microbiol* **67**: 426–433.
- Kimura, Y., Miyake, R., Tokumasu, Y., and Sato, M. (2000) Molecular cloning and characterization of two genes for the biotin carboxylase and carboxyltransferase subunits of acetyl coenzyme A carboxylase in *Myxococcus xanthus*. *J Bacteriol* **182**: 5462–5469.
- Klappenbach, J.A., and Pierson, B.K. (2004) Phylogenetic and physiological characterization of a filamentous anoxygenic photoautotrophic bacterium '*Candidatus Chlorothrix halophila*' gen. nov., sp. nov., recovered from hypersaline microbial mats. *Arch Microbiol* **181**: 17–25.
- Kumar, S., Tamura, K., and Nei, M. (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**: 150–163.
- Li, S.-J., and Cronan, J.E. (1992a) The gene encoding the biotin carboxylase subunit of *Escherichia coli* acetyl-CoA carboxylase. *J Biol Chem* **267**: 855–863.
- Li, S.-J., and Cronan, J.E. (1992b) The genes encoding the two carboxyltransferase subunits of *Escherichia coli* acetyl-CoA carboxylase. *J Biol Chem* **267**: 16841–16847.
- Lin, T.-W., Melgar, M.M., Kurth, D., Swamidass, S.J., Purdon, J., Tseng, T., *et al.* (2006) Structure-based inhibitor design of AccD5, an essential acyl-CoA carboxylase carboxyltransferase domain of *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* **103**: 3072–3077.
- Madigan, M.T., Peterson, S.R., and Brock, T.D. (1974) Nutritional studies on *Chloroflexus*, a filamentous photosynthetic, gliding bacterium. *Arch Microbiol* **100**: 97–103.
- Madigan, M.T., and Brock, T.D. (1977) CO₂ fixation in photosynthetically-grown *Chloroflexus aurantiacus*. *FEMS Microbiol Lett* **1**: 301–304.
- Madigan, M.T., Takigiku, R., Lee, R.G., Gest, H., and Hayes, J.M. (1989) Carbon isotope fractionation by thermophilic phototrophic sulfur bacteria: evidence for autotrophic growth in natural populations. *Appl Environ Microbiol* **55**: 639–644.
- Madigan, M.T., Jung, D.O., Karr, E.A., Sattley, W.M., Achenbach, L.A., and van der Meer, M.T.J. (2005) Diversity of anoxygenic phototrophs in contrasting extreme environments. In *Geothermal Biology and Geochemistry in Yellowstone National Park*. Inskeep, W.P., and McDermott, T.R. (eds). Bozeman: Montana State University Publications, pp. 203–219.
- Marini, P., Li, S.-J., Gardiol, D., Cronan, J.E., and de Mendoza, D. (1995) The genes encoding the biotin carboxyl carrier protein and biotin carboxylase subunits of *Bacillus subtilis* acetyl coenzyme A carboxylase, the first enzyme of fatty acid synthesis. *J Bacteriol* **177**: 7003–7006.
- van der Meer, M.T.J., Schouten, S., de Leeuw, J.W., and Ward, D.M. (2000) Autotrophy of green non-sulphur bac-

- teria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. *Environ Microbiol* **2**: 428–435.
- van der Meer, M.T.J., Schouten, S., van Dongen, B.E., Rijpstra, W.I.C., Fuchs, G., Sinninghe Damsté, J.S., *et al.* (2001) Biosynthetic controls on the ^{13}C -contents of organic components in the photoautotrophic bacterium *Chloroflexus aurantiacus*. *J Biol Chem* **276**: 10971–10976.
- van der Meer, M.T.J., Schouten, S., Hanada, S., Hopmans, E.C., Sinninghe-Damaste, J.S., and Ward, D.M. (2002) Alkane-1,2-diol based glycosides and fatty glycosides in *Roseiflexus castenholzii* and hot spring microbial mats. *Arch Microbiol* **178**: 229–237.
- van der Meer, M.T.J., Schouten, S., Sinninghe Damsté, J.S., de Leeuw, J.W., and Ward, D.M. (2003) Compound-specific isotopic fractionation patterns suggest different carbon metabolisms among *Chloroflexus*-like bacteria in hot-spring microbial mats. *Appl Environ Microbiol* **69**: 6000–6006.
- van der Meer, M.T.J., Schouten, S., Bateson, M.M., Nübel, U., Wieland, A., Kühl, M., *et al.* (2005) Diel variations in carbon metabolism by green nonsulfur-like bacteria in alkaline siliceous hot spring microbial mats from Yellowstone National Park. *Appl Environ Microbiol* **71**: 3978–3986.
- Menendez, C., Bauer, Z., Huber, H., Gad'on, N., Stetter, K.-O., and Fuchs, G. (1999) Presence of acetyl coenzyme A (CoA) carboxylase and propionyl-CoA carboxylase in autotrophic *Crenarchaeota* and indication for operation of a 3-hydroxypropionate cycle in autotrophic carbon fixation. *J Bacteriol* **181**: 1088–1098.
- Nübel, U., Bateson, M.M., Vandieken, V., Wieland, A., Kühl, M., and Ward, D.M. (2002) Microscopic examination of distribution and phenotypic properties of phylogenetically diverse *Chloroflexaceae*-related bacteria in hot spring microbial mats. *Appl Environ Microbiol* **68**: 4593–4603.
- Pierson, B.K., and Castenholz, R.W. (1974a) Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacteria. *Arch Microbiol* **100**: 283–305.
- Pierson, B.K., and Castenholz, R.W. (1974b) A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. *Arch Microbiol* **100**: 5–24.
- Raymond, J. (2005) The evolution of biological carbon and nitrogen cycling – a genomic perspective. *Rev Mineral Geochem* **59**: 211–231.
- Rodríguez, E., and Gramajo, H. (1999) Genetic and biochemical characterization of the α and β components of a propionyl-CoA carboxylase complex of *Streptomyces coelicolor* A3(2). *Microbiology* **145**: 3109–3119.
- Rodríguez, E., Banchio, C., Diacovich, L., Bibb, M.J., and Gramajo, H. (2001) Role of an essential acyl coenzyme A carboxylase in the primary and secondary metabolism of *Streptomyces coelicolor* A3(2). *Appl Environ Microbiol* **67**: 4166–4176.
- Samols, D., Thorton, C.G., Murtif, V.L., Kumar, G.K., Haase, F.C., and Wood, H.G. (1988) Evolutionary conservation among biotin enzymes. *J Biol Chem* **263**: 6461–6464.
- Sandbeck, K.A., and Ward, D.M. (1981) Fate of immediate methane precursors in low sulfate hot spring algal-bacterial mats. *Appl Environ Microbiol* **41**: 775–782.
- Sirevåg, R., and Castenholz, R. (1979) Aspects of carbon metabolism in *Chloroflexus*. *Arch Microbiol* **120**: 151–153.
- Steunou, A.-S., Bhaya, D., Bateson, M.M., Melendrez, M.C., Ward, D.M., Brecht, E., *et al.* (2006) *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. *Proc Natl Acad Sci USA* **103**: 2398–2403.
- Strauss, G., and Fuchs, G. (1993) Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *Eur J Biochem* **215**: 633–643.
- Swingle, W.D., Sadekar, S., Mastrian, S.D., Matthies, H.J., Hao, J., Ramos, H., *et al.* (2007) The complete genome sequence of *Roseobacter denitrificans* reveals a mixotrophic rather than photosynthetic metabolism. *J Bacteriol* **189**: 683–690.
- Ugo'kova, N.V., and Ivanovsky, R.N. (2000) On the mechanism of autotrophic fixation of CO_2 by *Chloroflexus aurantiacus*. *Mikrobiologiya (English translation)* **69**: 139–142.
- Vignais, P.M., Billoud, B., and Meyer, J. (2001) Classification and phylogeny of hydrogenases. *FEMS Microbiol Rev* **25**: 455–501.
- Ward, D.M., Tayne, T.A., Anderson, K.L., and Bateson, M.M. (1987) Community structure, and interactions among community members in hot spring cyanobacterial mats. *Symp Soc Gen Microbiol* **41**: 179–210.
- Ward, D.M., Bateson, M.M., Ferris, M.J., and Nold, S.C. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol Mol Biol Rev* **62**: 1353–1370.
- Ward, D.M., Bateson, M.M., Ferris, M.J., Kühl, M., Wieland, A., Koeppl, A., and Cohan, F.M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. *Philos Trans R Soc Lond B* **361**: 1997–2008.

Supplementary material

The following supplementary material is available for this article online:

Supplementary text. Metagenome methods.

Table S1. Putative 3-OHP genes and their corresponding hidden Markov model hits.

Table S2. Metagenome libraries.

This material is available as part of the online article from <http://www.blackwell-synergy.com>