Candidatus Chloracidobacterium thermophilum: An Aerobic Phototrophic Acidobacterium
Donald A. Bryant, et al.
Science 317, 523 (2007);
DOI: 10.1126/science.1143236

The following resources related to this article are available online at www.sciencemag.org (this information is current as of July 27, 2007):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/317/5837/523

Supporting Online Material can be found at:
http://www.sciencemag.org/cgi/content/full/317/5837/523/DC1

This article cites 17 articles, 7 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/317/5837/523#otherarticles

Information about obtaining reprints of this article or about obtaining permission to reproduce this article in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl
farmers of the Fertile Crescent domesticated grains and cereals as well as livestock (1, 3, 4, 30–32). In parallel, the endemic wildcats of the region may have adapted by both regulating the rodents in the grain stores and abandoning their aggressive wild-born behaviors. The archaeological imprints left in the genomes of living cats here weigh into inferences about the timing, steps, and provenance of domestication—a dynamic exercise depicted in art, in history, and in human cultural development since recorded evidence began.

References and Notes
28. See supporting material on Science Online.
33. We thank M. W. Smith, A. Schmidt-Kuntzel, C. O’Higgins, and B. Gold for discussions and J. Bruskich, A. Brandt, S. Rosendale, and F. Hussain for technical assistance. We appreciate the efforts of all of our collaborators listed in fig. S1 who provided biological specimens used in this study. All tissues were collected in full compliance with federal fish and wildlife permits (Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) issued to the National Cancer Institute (NCI) principal officer, S.J.O) by the Fish and Wildlife Service, U.S. Department of the Interior. Supported by NCI grant N01-BC-12400 and the Intramural Research Program of the NCI Center for Cancer Research. Sequences have been deposited in GenBank with accession numbers EF587016 to EF587179.

### Candidatus Chloracidobacterium thermophilum: An Aerobic Phototrophic Acidobacterium


Only five bacterial phyla with members capable of chlorophyll (Chl)–based phototrophy are presently known. Metagenomic data from the phototrophic microbial mats of alkaline siliceous hot springs in Yellowstone National Park revealed the existence of a distinctive bacteriochlorophyll (BChl)–synthesizing, phototrophic bacterium. A highly enriched culture of this bacterium grew photolithotrophically, synthesized BChls a and c under oxidative conditions, and had chlorosomes and type 1 reaction centers. "Candidatus Chloracidobacterium thermophilum" is a BChl-producing member of the poorly characterized phylum Acidobacteria.

Sequecing environmental DNA is a powerful approach for predicting the physiological and metabolic potential of microbial ecosystems. Metagenomic analyses have provided insights into the properties of uncultured microorganisms that have escaped detection in field studies (1–6). We used metagenomic data from the microbial mat communities...
of Octopus and Mushroom Springs in Yellowstone National Park (Yellowstone NP) (5–7) to search for previously unrecognized BCHl/Chl-synthesizing phototrophs (chlorophototrophs).

Only five bacterial phyla contain chlorophototrophs: Cyanobacteria, Chlorobi, Proteobacteria, Chloroflexi, and Firmicutes (8, 9). In chlorophototrophs, light energy is transduced into chemical potential energy by reaction centers, photo-oxidoreductases that form two families of BCHl/Chl-containing, pigment-protein complexes (10). Type 1 reaction centers include cyanobacterial Photosystem I and the homodimeric reaction centers of Chlorobi and heliobacteria (Firmicutes). Type 2 reaction centers include cyanobacterial Photosystem II and the reaction centers of Proteobacteria and Chloroflexi. Although their subunits are not discernibly similar in sequence, the two reaction-center types probably share a common evolutionary origin because their electron-transfer domains have similar structures and cofactor arrangements (11).

16S ribosomal RNA (rRNA) surveys of the Yellowstone NP phototrophic mat communities suggested the presence of green sulfur bacteria (12). Metagenomic data obtained from these mats (5–7, 9) were queried with the tblastn algorithm and the amino acid sequence of PscA, the BCHl a-binding apoprotein of a homodimeric, P840-binding, type 1 reaction center from Chlorobium tepidum (9, 13). Two incomplete sequences that encode (B)Chl-binding apoproteins of type 1 reaction centers were recovered. Phylogenetic analyses suggested that one sequence (OS GSB PscA) belonged to a green sulfur bacterium that grouped with PscA from Chlororhopterus thalassium (Fig. 1A). The second sequence, labeled Cab. thermosthaliuim PscA, was only very distantly related to other PscA sequences and other type 1 reaction-center proteins (Fig. 1A and figs. S1 to S3); this suggested the existence of a previously unrecognized chlorophototroph in the mat community. Plasmids encoding this gene were recovered and sequenced (9), and the data revealed a probable operon, 5’-pscAB-fmoA (Fig. 1B). pscB encodes the apoprotein of an 8Fe-8S ferredoxin of a type 1 reaction center (13), and pmoA encodes the BCHl a-binding, Fenna-Matthews-Olson protein (Fig. 1B) and figs. S4 and S5) (14). The pscA gene predicted a protein of 865 amino acids that was larger than the apoproteins of other type 1 reaction centers. Most of the size difference was caused by an insertion of ~165 amino acids into a periplasmic loop between transmembrane α helices VII and VIII (Fig. 1C and fig. S1). Many of the genes flanking the pscAB-fmoA operon predicted proteins that were most similar to those of Acidobacterium sp. Elfin345 or Solibacter usitatus Elfin6076, two soil bacteria belonging to the poorly characterized phylum Acidobacteria, whose genomes were recently sequenced. Thus, we hypothesized that the unknown phototroph might belong to the phylum Acidobacteria.

Analyses of metagenomic sequence data and end-reads from a bacterial artificial chromosome (BAC) library predicted that the pscAB-fmoA, recA, and rRNA genes were encoded on a single BAC insert (9). We confirmed this prediction by sequencing the 271,846-base pair (bp) insert (fig. S6; GenBank accession number EF531339).

Fig. 1. (A) Unrooted neighboring tree of protein sequences for type 1 reaction centers from Cyanobacteria, Chlorobi, heliobacteria, and a divergent PscA-like sequence (Cab. thermosthellium) obtained from the metagenome of Octopus and Mushroom Springs, Yellowstone NP. Node mark with a “*” have 100% bootstrap support; the node separating the PsaA and PsaB clades is marked with a “o” and has 70% bootstrap support. (B) Organization of the psaB, pscB, and fmoA genes in Cab. thermosthellium. (C) Diagram showing the organization of the 11 predicted transmembrane α helices of Cab. thermosthellium PsA. “Fx” denotes the positions of two cysteine residues that are predicted to be ligands to the intersubunit [4Fe-4S] cluster Fx, and “P” indicates the approximate position of the conserved histidine residue predicted to form a ligand to one of the two predicted BCHl a molecules of the reaction-center special pair. Green Roman numerals I to VI indicate the antenna domain, and blue Roman numerals VII to XI indicate the electron-transfer domain.
which forms the baseplate of chlorosomes, the light-harvesting antenna complexes in Chlorobi and Chloroflexi (17), was also found. The presence of genes encoding subunits of a nicotinamide adenine dinucleotide, reduced (NADH): quinone oxidoreductase, the quinol:cytochrome c oxidoreductase, and cytochrome oxidase implied that the acidobacterium might respire aerobically. The data collectively predicted that the mat acidobacterium is probably an aerobic photoheterotroph that synthesizes BCHl a, methylated BCHl c, chlorosomes, FmoA, and type 1 reaction centers. The organism seemed physiologically most similar to aerobic anoxygenic phototrophs or to facultatively photo(auto/hetero) trophic organisms like Roseiflexus and Chloroflexus spp. (8).

Allewalt et al. (18) described the establishment of uni-cyanobacterial enrichments for thermophilic Synechococcus spp. from Octopus Spring (Fig. 2A). The presence of a closely related acidobacterium in an oxic enrichment was indicated by specific polymerase chain reaction (PCR) amplification of genes for acidobacterial pscA, fmoA, csmA, acsF, bchU, recA, and 16S rRNA (9) (table S1). By serially culturing this enrichment culture in a modified cyanobacterial growth medium containing ammonium, a mixture of carbon sources, and the Photosystem II inhibitor atrazine, we eliminated Synechococcus sp. strain JA-2-3B’ a (2-13). The resulting brownish-orange culture contained only the acidobacterium and Anoxybacillus sp. (Fig. 2A).

The latter could be isolated on Luria-Bertani plates, did not synthesize BCHl (Fig. 2A), and did not possess any genes for BCHl biosynthesis or formation of a light-harvesting apparatus. The absorption spectrum of the enrichment culture shows a maximum at 743 to 745 nm that is characteristic of BCHl c aggregates in chlorosomes (Fig. 2B). Using a method for the isolation of chlorosomes from green sulfur bacteria (9), we isolated chlorosomes that were morphologically similar to those of C. tepidum (Fig. 3). Serial culturing of the Anoxybacillus sp.--acidobacterium enrichment in the dark resulted in the simultaneous loss of the 746-nm absorbance (Fig. 2B) and acidobacterial 16S rRNA (Fig. 2C). This experiment definitively establishes that the acidobacterium grows photoheterotrophically. The enrichment did not grow with BCHl a as the sole carbon source.

High-performance liquid chromatography (HPLC) analyses of pigments extracted from cells from the Anoxybacillus sp.–acidobacterium enrichment verified the presence of both BCHl c and BCHl a (Fig. 4). The complex pattern of BCHl c homologs, appearing in groupings of four (Fig. S13), was consistent with methylation of both the C-82 and C-121 carbons, as occurs in C. tepidum and other Chlorobi strains (19). The elution profile also indicated that BCHl c was esterified by several alcohol species. Only trace amounts of farnesylated BCHl c were detected, and the major BCHl c homologs were more hydrophobic than farnesylated BCHl c, the esterifying alcohol most commonly found in green sulfur bacteria (Fig. 4). The major BCHl c homologs were slightly more hydrophobic than the BCHl c homologs produced by Chloroflexus aurantiacus Y-400-1 (Fig. 4). However, Chloroflexus spp. do not methylate BCHl c at the C-82 or C-121 positions, and their BCHl c is typically esterified with multiple alcohols including phytol, geranylgeraniol, and stearol (8, 19).

16S rRNA analyses, including the present study, indicate that the acidobacterial chlorophototroph grows at temperatures from ~50° to 66°C at Mushroom Spring, Octopus Spring, and Green Finger Pool (15, 20). 16S rRNA sequences closely related to that of this acidobacterium have also been recovered from Mammoth Hot Springs in Yellowstone NP and from hot springs in Tibet and Thailand (21, 22). Therefore, acidobacterial chlorophototrophs may be members of microbial mat communities associated with thermal features worldwide. Because strains of Acidobacteria are also widely distributed in soils and other environments (15, 23), it will be interesting to determine whether phototrophy is widespread in this poorly characterized phylum. Analyses of the genomes of two acidobacteria, S. isitanus Ellin6076 and Acidobacterium sp. Ellin345, demonstrate that these organisms do not have this capability (24).

In this study we applied metagenomics to discover a previously unknown chlorophototroph, and we used enrichment techniques and biochemical methods to verify that this organism is a bacillus (Fig. 2, D and E) that synthesizes BCHls.

Fig. 2. (A) Cultures containing (1) Synechococcus sp. strain JA-2-3B’ a (2-13), Cab. Thermophilum, and Anoxybacillus sp.; (2) Cab. thermophilum and Anoxybacillus sp.; and (3) Anoxybacillus sp. (B) Absorption spectra of an enrichment culture containing Cab. thermophilum and Anoxybacillus sp. after serial culturing three times in the light (L3) or in the dark (D3). The 745-nm absorption due to aggregated BCHl c is only observed in the light-grown cells. Note the change from 746 to 745 nm to enhance BCHl c fluorescence (9).

Fig. 3. Transmission electron micrograph of isolated chlorosomes from an enrichment culture containing Cab. thermophilum and Anoxybacillus sp. after negative staining with 1% (w/v) uranyl acetate.
a and c produces chlorosomes underoxic conditions. Although chlorosomes are also found in some Chloroflexi, the new chlorophototroph is the only described organism outside the phylum Chlorobi that produces FmoA. Growth of this acidobacterium is strongly stimulated by light under phototrophic conditions, but additional studies will be required to establish whether the organism is capable of autotrophic growth. Because no chlorophototroph with these properties has yet been described, we propose the name “Candidatus Chloracidobacterium thermophilum,” gen. nov., sp. nov., for this BChl-synthesizing, phototrophic member of the phylum Acidobacteria.

References and Notes
9. Supporting material is available on Science Online.
25. This work was supported by NSF grant MCB-0523100 and U.S. Department of Energy grant DE-FG02-94ER20137 (to D.A.B.). The metagenomics database used in this work was created under the auspices of grant EF-0328698 from the Frontiers in Integrative Biology Program of the NSF (to D.M.W.), who also acknowledges support from the NASA Exobiology Program (NAGS-8824). D.A.B. thanks K. Schuster, J. Eisen, R. Blankenship, R. Casterholz, and S. Giovannoni for helpful discussions and comments on the manuscript. D.A.B. and D.M.W. also gratefully acknowledge the Thermal Biology Institute of Montana State University (NASA NAGS-8807) for support and hospitality while D.A.B. was a visiting fellow in summer 2005, when this work was initiated. We thank M. Melendrez and Q. Tao for preparation of the BAC library. The pscAB-fmoA, recA, and rRNA genes were encoded on a single BAC clone, M60-018 J19: GenBank accession number EF531339. Rosellefexus sp. isolate, strain RS1: GenBank accession numbers AAQ00000000. Synecococcus sp. OS-A and OS-B: genome sequences: GenBank accession numbers CP002039 and CP000240.

Supporting Online Material
www.sciencemag.org/cgi/content/full/317/5837/523/DC1
Methods
SOM Text
Figs. S1 to S13
Table S1
References
30 March 2007; accepted 27 June 2007
10.1126/science.1143236

Noise in Gene Expression Determines Cell Fate in Bacillus subtilis
Hédia Maamart, Arjun Raj, David Dubnau†

Random cell-to-cell variations in gene expression within an isogenic population can lead to transitions between alternative states of gene expression. Little is known about how these variations (noise) in natural systems affect such transitions. In Bacillus subtilis, noise in ComK, the protein that regulates competence for DNA uptake, is thought to cause cells to transition to the competent state in which genes encoding DNA uptake proteins are expressed. We demonstrate that noise in ComK expression selects cells for competence and that experimental reduction of this noise decreases the number of competent cells. We also show that transitions are limited temporally by a reduction in ComK transcription. These results illustrate how such stochastic transitions are regulated in a natural system and suggest that noise characteristics are subject to evolutionary forces.

Variability in gene expression within a population of genetically identical cells enables those cells to maintain a diversity of phenotypes, potentially enhancing fitness (1, 2). When the underlying gene network contains regulatory positive feedback loops, indi-