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SHORT COMMUNICATIONS

Phylogenetic Position of Three Strains of Green Sulfur Bacteria

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Green sulfur bacteria (family *Chlorobiaceae*) are a physiologically homogeneous group forming a separate phylogenetic branch isolated from other bacteria (phylum *Chlorobi*). Until recently, their taxonomy was based on phenotypic characteristics (morphological and biochemical properties) [1, 2]. Comparative analysis of the sequences of the 16S rRNA genes and the *fmo* genes, encoding FMO protein, which occurs exclusively in *Chlorobiaceae* representatives, led to substantial reorganization of the taxonomy of this group of bacteria [3]. As a result, strains previously assigned to one species may have been transferred to several different species and even genera.

The culture collection of the Department of Microbiology, Moscow State University, includes several strains of green sulfur bacteria previously assigned to *Chl. limicola* forma *thiosulfatophilum* (strains C, X, and L, or KM MGU 319, 320, and 321, respectively). These have been used in studies of various aspects of metabolism of green sulfur bacteria [4–8]; therefore, determination of their phylogenetic positions by chemotaxonomic and molecular biological methods is topical.

Using the method described in [9], we determined the fatty acid compositions of strains C, X, and L (table); such data are rather informative but scarce for green sulfur bacteria. Strains L, X, and C were found to contain a considerable amount of palmitoleic (16:1ω7), palmitic (16:0) and myristic (14:0) acids, whereas the 18:1 acids (oleic and *cis*-vaccenic) were virtually absent. Such a fatty acid pattern is characteristic of *Chlorobiaceae* representatives [2, 3, 10].

In order to perform comparative analysis of 16S rRNA genes, their fragments were amplified using standard bacterial primers as described in [11]. As a result, sequences of more than 1300 nucleotides were determined for strains C, X, and L (and deposited in GenBank: *Chb. parvum* strain L, EF560699; *Chb. limnaeum* strain X, EF560700; and *Chb. limnaeum* strain C, EF560701).

According to the preliminary phylogenetic screening performed with BLAST (http://www.ncbi.nlm.nih.gov/blast), all the strains studied belonged to the subdivision of green sulfur bacteria. For more detailed phylogenetic analysis, the 16S rRNA gene sequences of the strains studied and reference bacterial species were aligned using the CLUSTLW v1.75 program. Phylogenetic trees were constructed using different algorithms implemented in the TREECONW software package [12]. In the constructed phylogenetic tree (Fig. 1), all three strains studied fell into phylogenetic cluster 4,

Fatty acid composition (% of total) in cells of strains C, L, and X

Fatty acids	Strain L	Strain X	Strain C
12:0	2.8	1.3	1.8
14:1	0.3	3.1	2.3
14:0	23.6	31.4	30.4
15:0	0.7	0.0	0.3
16:1ω7	52.8	48.3	46.5
16:1ω5	1.2	2.3	1.7
16:0	18.5	12.7	16.6
18:1ω9	0.0	0.4	0.3
18:1ω7	0.0	0.0	0.0
18:0	0.0	0.4	0.0

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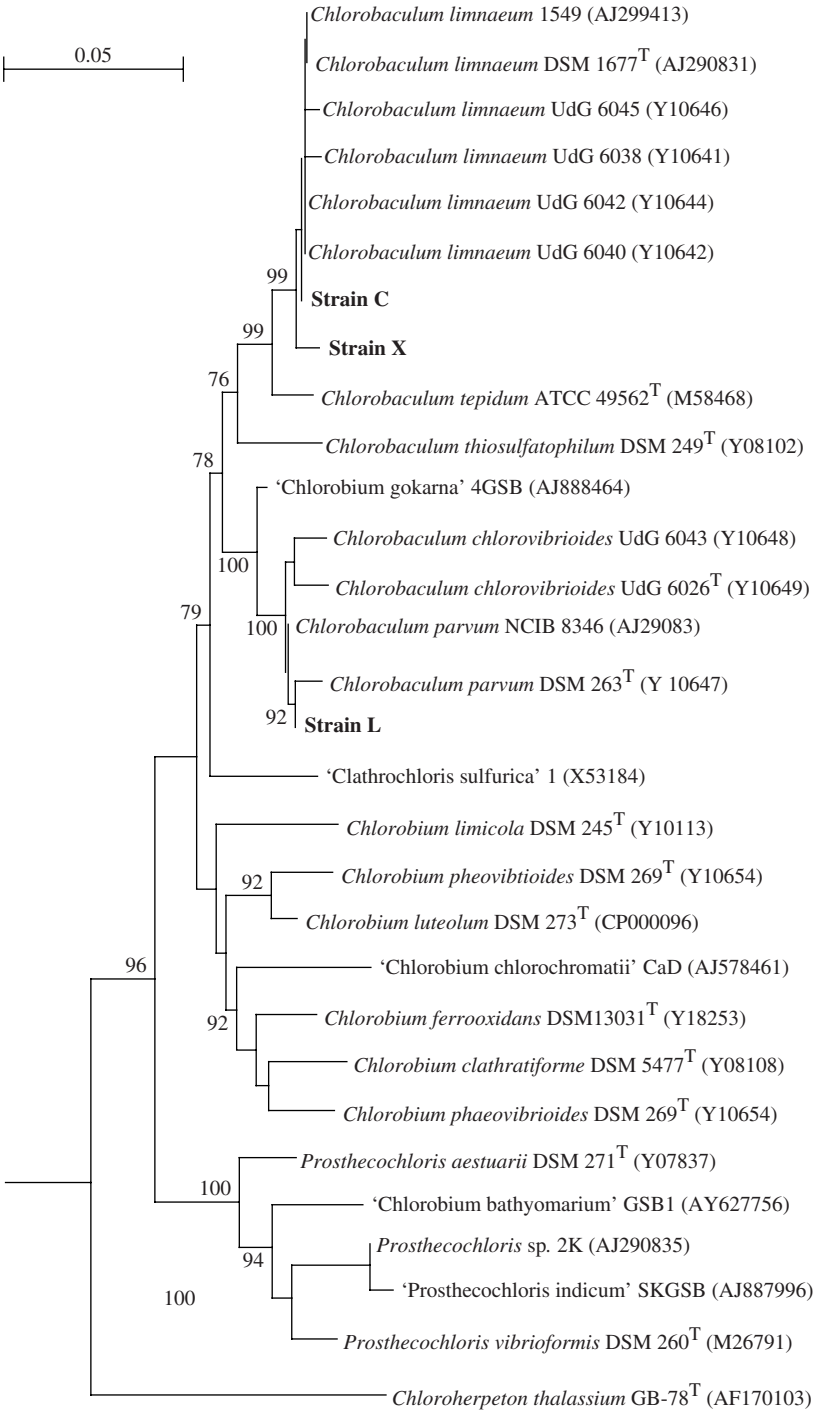

Fig. 1. Position of strains C, L, and X within the family *Chlorobiaceae* in the tree constructed based on the analysis of 16S rRNA gene nucleotide sequences using the neighbor-joining algorithm. The scale bar corresponds to 5 nucleotide substitutions per 100 nucleotides (evolutionary distances). The numerals show the statistical significance of the branching order as determined with bootstrap analysis of 100 alternative trees (values higher than 70% were considered significant).

which has recently been singled out to form the new genus *Chlorobaculum* [3]. Strain L was close (99.4% 16S rRNA identity) to strain DSM 263, formerly called *Chl. vibrioforme* f. *thiosulfatophilum* and renamed *Chb. parvum*, as well as to NCIB 8346, another strain of this species (99.8%). The high level of similarity between the 16S rRNA gene sequences gave evidence of the possibility of affiliation of strain L with *Chb. parvum.*

16S rRNA gene sequences of strains X and C were virtually identical (99.1% identity) and fell into the

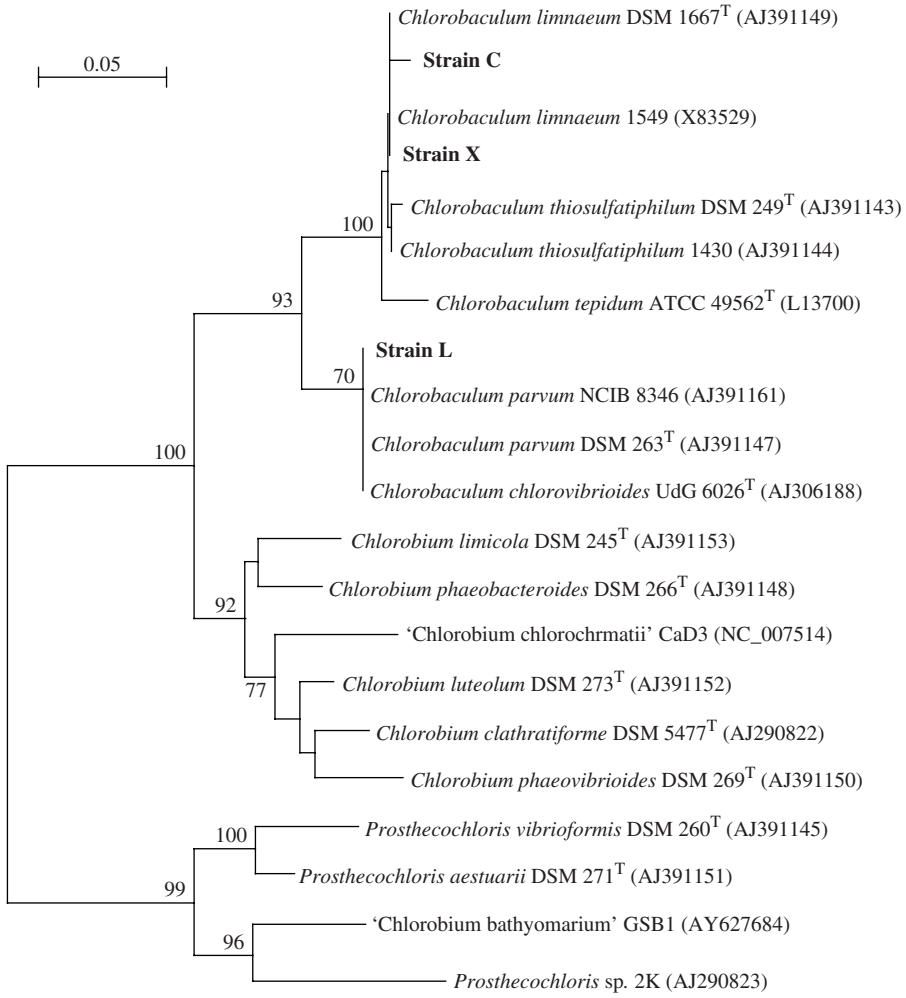

Fig. 2. Position of strains C, L, and X within the family *Chlorobiaceae* in the tree constructed based on the analysis of inferred FMO protein amino acid sequences. The neighbor-joining algorithm was used. The sequences determined in this study are bold-typed. The numerals show the significance of branching order as determined with bootstrap analysis (values higher than 70% were considered significant). The scale bar shows the evolutionary distance corresponding to 5 substitutions per 100 amino acid residues.

subcluster of *Chb. limnaeum* strains, exhibiting a high level of similarity to them (98.6–99.8%). This indicated the possibility of identifying strains X and C with the species *Chb. limnaeum*.

To confirm the inferences from rRNA trees, we additionally determined the nucleotide sequences of *fmo* gene fragments of the studied strains by the method described in [13]. The nucleotide sequences of the *fmo* gene fragments thus obtained were deposited in the GenBank database: *Chb. parvum* strain L, EF560704; *Chb. limnaeum* strain X, EF560703; and *Chb. limnaeum* strain C, EF560702. The determined *fmo* gene sequences were conceptually translated into amino acid sequences, which were aligned with the corresponding sequences of *Chlorobiaceae* representatives. Not only did the results of this phylogenetic analysis agree with the results of the analysis of 16S rRNA gene sequences, but they also allowed more exact identification the studied strains.

In the constructed phylogenetic tree (Fig. 2), all the strains under study fell into the genus *Chlorobaculum.* The *fmo* gene sequences of strain L and strains of the species *Chb. parvum* and *Chb. chlorovibrioides* were 100% identical, which, in the aggregate with the results of analysis of 16S rRNA genes, allows unambiguous identification of strain L as a representative of the species *Chb. parvum.* The *fmo* gene sequences of strains C and X were close to each other and to the analogous sequences of *Chb. limnaeum* strains (98.6–100% identity), which confirmed the affiliation of strains C and X with *Chb. limnaeum.*

Thus, based on a complex phylogenetic study, strains C and X are assigned to the species *Chb. limnaeum* and strain L is attributed to *Chb. parvum*.

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REFERENCES

1. Pfennig, N., Green Sulfur Bacteria, *Bergey's Manual of Systematics Bacteriology*, 1st ed., vol. 1, Staley, J.T., Bryant, M.P., Pfennig, N., and Holt, J.G., Eds., Baltimore: Williams & Wilkins, 1989, pp. 1697–1709.
2. Overmann, J., Green Sulfur Bacteria, *Bergey's Manual of Systematics Bacteriology*, 2nd ed., vol. 1, Boone, D.R., Castenholz, R.W., and Garrity, G.M., Eds., New York: Springer, 2001, pp. 601–605.
3. Imhoff, J.F., Phylogenetic Taxonomy of the Family *Chlorobiaceae* on the Basis of 16S rRNA and FMO (Fenna–Matthews–Olson Protein) Gene Sequences, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 941–951.
4. Ivanovsky, R.N., Sintsov, N.N., and Kondratieva, E.N., ATP-Linked Citrate Lyase Activity in the Green Sulfur Bacterium *Chlorobium limicola* forma *Thiosulfatophilum, Arch. Microbiol.*, 1980, vol. 128, pp. 239–241.
5. Keppen, O.I., Lebedeva, N.V., Petukhov, S.A., and Rodionov, Yu.V., Nitrogenase Activity in the Green Sulfur Bacterium *Chlorobium limicola, Mikrobiologiya*, 1985, vol. 54, pp. 36–41.
6. Rodionov, Y.V., Lebedeva, N.V., and Kondratieva, E.N., Ammonia Inhibition of Nitrogenase Activity in Purple and Green Bacteria, *Arch. Microbiol.*, 1986, vol. 143, pp. 345–347.
7. Serebryakova, L.T., Zorin, N.A., and Gogotov, I.N., Hydrogenase of the Green Sulfur Bacterium *Chlorobium limicola* forma *thiosulfatophilum, Biokhimiya*, 1987, vol. 52, pp. 908–914.
8. Lebedeva, N.V., Malinina, N.V., and Ivanovsky, R.N., A Comparative Study of the Isocitrate Dehydrogenases of *Chlorobium limicola* forma *thiosulfatophilum* and *Rhodopseudomonas palustris, Mikrobiologiya*, 2002, vol. 71, no. 6, pp. 762–767 [*Microbiology* (Engl. Transl.), vol. 71, no. 6, pp. 657–661].
9. Zhilina, T.N., Zavarzin, G.A., Rainey, F.A., Pikuta, E.V., Osipov, G.A., and Kostrikina, N.A., *Desulfonatronovibrio hydrogenovorans* gen. nov., an Alkaliphilic Sulfate-Reducing Bacterium, *Int. J. Syst. Bacteriol.*, 1997, vol. 47, pp. 144–149.
10. Kenyon, C.N., Complex Lipids and Fatty Acids of Photosynthetic Bacteria, *The Photosynthetic Bacteria*, Clayton, R. and Sistrom W., Eds., New York, London: Plenum, 1978, pp. 281–313.
11. Tourova, T.P., Spiridonova, E.M., Slobodova, N.V., Boulygina, E.S., Keppen, O.I., Kuznetsov, B.B., and Ivanovsky, R.N., Phylogeny of Anoxygenic Filamentous Phototrophic Bacteria of the Family *Oscillochloridaceae* as Inferred from Comparative Analyses of the *rrs, cbbL*, and *nifH* Genes, *Mikrobiologiya*, 2006, vol. 75, no. 2, pp. 235–244 [*Microbiology* (Engl. Transl.), vol. 75, no. 2, pp. 192–200].
12. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Applic. Biosci.*, 1994, vol. 10, pp. 569–570.
13. Alexander, B., Andersen, J.H., Cox, R.P., and Imhoff, J.F., Phylogeny of Green Sulfur Bacteria on the Basis of Gene Sequences of 16S rRNA and of the Fenna–Matthews–Olson Protein, *Arch. Microbiol.*, 2002, vol. 178, pp. 131–140.