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EXPERIMENTAL ARTICLES

Comparative Analysis of the N-Terminal Sequence of *Geobacter sulfurreducens* AM-1 Methacrylate Reductase

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Abstract—Comparative analyses of the amino acid sequence of the N-terminus of methacrylate reductase (50 kDa) from the anaerobic bacterium *Geobacter sulfurreducens* AM-1 revealed significant similarity to the sites of flavocytochromes *c* and flavin-containing proteins of anaerobic bacteria from the genera *Anaeromyxobacter, Campylobacter, Desulfatibacillum, Desulfuromonas, Geobacter, Parasutterella, Shewanella, Sulfurospirillum, Sutterella*, and *Wolinella* (belonging to four classes of the *Proteobacteria*), *Denitrovibrio* of the phylum *Deferribacteres, Desulfitobacterium* of the phylum *Firmicutes, Eggerthella* and *Slackia* of the phylum *Actinobacteria*, and *Spirochaeta* of the phylum *Spirochaetes*. High homology was also revealed between the methacrylate reductase amino acid sequence and the flavoproteins of aerobic bacteria *Frankia* and *Gordonia* from the phylum *Actinobacteria* and of the archaeon *Haloterrigena turkmenica* from the phylum *Euryarchaeota*. Among 11 *Geobacter* strains with known genomes, only *G. lovleyi* SZ capable of chlororespiration was found to contain an amino acid fragment of flavocytochrome *c* with pronounced similarity (80%) to the N-terminus of methacrylate reductase. The physiological properties common to these bacteria are discussed. Molecular masses and the hypothetical functions and localization of the homologous proteins are analyzed. The grouping of proteins according to the phylogenetic affiliation of their owners is discussed. Hypotheses concerning the distribution and evolutionary role of such proteins in microorganisms are suggested.

Keywords: anaerobic respiration, methacrylate reductase, *Geobacter sulfurreducens* AM-1, *Proteobacteria*, N-terminus, BLAST, amino acid homology, flavocytochromes *c*, flavoproteins

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Members of the genus *Geobacter* (*Deltaproteobacteria*) are important agents in bioremediation of radioactive metals [1–4]. They play a significant role in the global cycles of carbon and metals, reducing Fe(III) to Fe(II) and U(VI) to U(IV), oxidizing acetate and other organic compounds, and participate in humus decomposition. Moreover, they are electrotrophs [5].

G. sulfurreducens AM-1 was isolated in the course of the investigation of the pathways for decomposition of methacrylate industry wastes [6]. It is capable of complete oxidation of an organic compound (acetate) coupled to reduction of the anthropogenic organic compound methacrylate (=2-methylpropenoate). Methacrylate acts as the terminal electron acceptor of the reductase chain: it receives reducing equivalents from the cytoplasmic TCA cycle via the membrane menaquinone with eight isoprenoid residues in its side chain [7]. Conversion of methacrylate to isobutyrate was shown to be catalyzed by the periplasmic flavin-containing methacrylate reductase (50 kDa) [7, 8]. Its activity depends on the periplasmic tetraheme cytochrome *c* (30 kDa), which is the physiological electron donor for this enzyme. The "electron carrier" from menaquinone to cytochrome *c* (30 kDa) remains unknown. Involvement of additional cytochrome(s) *c* in the process was suggested. Cytoplasmic cytochromes *c* (12.5 and 15.5 kDa) and the membrane cytochrome (67.6 kDa) are the possible candidates [9].

Cytochrome *c*-methacrylate oxidoreductase was isolated and purified, and its terminal amino acid sequence (27 residues) was determined; it contained mostly hydrophobic amino acids [7]. The highest amino acid homology (68%) was found (1999) with the FccA periplasmic protein from *Wolinella succinogenes* (*Epsilonproteobacteria*). The hypothesized physiological role of the *W. succinogenes* FccA protein was methacrylate and acrylate reduction [10]. Several years later, similarity was found between cytochrome *c*-methacrylate oxidoreductase (50 kDa) and the flavoproteins of various classes of proteobacteria [8]. The highest amino acid homology was observed for flavoproteins of the gammaproteobacterium *Shewanella*

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oneidensis, NP_719167.1 (83%) and NP_717134.1 (75%).

The recent intense development of information technologies resulted in the sequencing of numerous bacterial genes and genomes. The number of the known complete bacterial genomic sequences increases faster than the rate of their annotation and publishing. For example, while the databases of the National Center for Biotechnology Information contain the genomes of 11 *Geobacter* species, only 5 have been described in the literature [11–15]. Therefore, it was suggested that comparison of the known methacrylate reductase fragment with the amino acid sequences from the databases could provide new results.

The goal of the present work was to analyze the distribution of the determined amino acid sequence of the unique methacrylate reductase from *G. sulfurreducens* among bacterial taxa, as well as the functions, localization, origin, and phylogenetic relation of the relevant proteins.

MATERIALS AND METHODS

Subject of investigation was the anaerobic bacterium *G. sulfurreducens* AM-1 from the culture collection of the Laboratory of Anaerobic Microorganisms, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences.

Object of investigation was the N-terminal amino acid sequence of the *G. sulfurreducens* AM-1 methacrylate reductase, which was previously determined [7].

All amino acid sequences of the proteins, as well as the nucleotide sequences of the genes of the enzymes and 16S rRNA, are available in the databases: GenBank, Gene, Genome, Nucleotide, and Protein from the server of the National Center for Biotechnology Information, National Library of Medicine, United States (NCBI; http://www.ncbi.nlm.nih.gov).

Comparative analysis of amino acid sequences was carried out using the PSI-BLAST program [16] from the NCBI server.

Detection of the signal peptide sequence was carried out using the PRED-TAT program [17] available at the server of the Department of Computer Science and Biomedical Informatics, University of Central Greece, Lamia, Greece (http://www.compgen.org).

Phylogenetic analysis of the 16S rRNA genes and the genes of the homologous proteins was carried out using the MEGA 4.0 software package [18]. Relations between the sequences were analyzed using the CLUSTALW program implemented in the MEGA package. Phylogenetic trees were constructed using the neighbor joining method and confirmed by bootstrap analysis (1000 replacements).

RESULTS

Comparative analysis of the N-terminal amino acid sequence of methacrylate reductase (50 kDa) of the anaerobic bacterium *G. sulfurreducens* AM-1 revealed pronounced ($E \leq 10^{-4}$) similarity to the sites of over 200 amino acid sequences. The table presents the amino acid sequences with the highest similarity to the compared one. These are mostly flavocytochromes *c* and flavin-containing succinate dehydrogenases and fumarate reductases of anaerobic and aerobic members of the *Bacteria* domain belonging to four classes of the *Proteobacteria* (10 genera), class *Actinobacteria*, phylum *Actinobacteria* (4 genera), phylum *Deferribacteres* (*Denitrovibrio*), phylum *Firmicutes* (*Desulfitobacterium*), phylum *Spirochaetes* (*Spirochaeta*), and of the aerobic member of the *Archaea* domain, class *Euryarchaeota* (*Haloterrigena turkmenica*) (table). In the case when a number of similar proteins was found in a single bacterial species (e.g., in the genomes of *Parasutterella excrementihominis* YIT 11859 and *Sutterella wadsworthensis* 3_1_45B), only one sequence was presented in the table, with their total number given in the "Additional similar sequences" column.

The table demonstrates that most of the protein sequences detected were 510–520 amino acid residues long, with molecular masses of the potential products (calculated from the number of amino acids) usually about 53–56 kDa, which is in agreement with the previously experimentally determined molecular mass of methacrylate reductase [7].

The homologous sequence was located at the N-terminal end of the protein molecule or near (30–40 amino acids) it. Since the native methacrylate reductase is a periplasmic protein (probably possessing a cleavable signal sequence for translocation), existence of a similar signal sequence was logical to expect in the protein homologues. The homologous proteins were tested using the PRED-TAT program [17] in order to search for two signal sequences: Tat (the longer and less hydrophobic one, containing two arginine residues, and transferring the protein after folding) and Sec (transferring proteins prior to development of their tertiary structure) (table). Existence of a cleavable signal sequence was predicted for 90% of the proteins: Tat peptides (27–45 amino acids, with two arginine residues at the 4th–13th positions of the cleavable peptide) in 77% of the proteins and Sec peptides in 13%.

Importantly, the proteins of different species both within a genus (*Anaeromyxobacter* and *Eggerthella*) and possibly of different genera and even different families (members of the order *Burkholderiales, P. excrementihominis* YIT 11859 and an unclassified microorganism *Burkholderiales* bacterium 1_1_47) exhibited high levels of similarity. In some cases, the proteins differed in one to five amino acids only. This was the case with flavocytochromes *c*

Distribution of the proteins with amino acid sequences exhibiting high similarity to the N-terminal end of the *Geobacter sulfurreducens* AM-1 methacrylate reductase

Domain	Class	Organism	Sequence no. in the database	Protein, function	Additional similar sequences	Superposition area, % of the total fragment length	Identity/ similarity, % of the total fragment length	E	Calculated Mr, Da	Possible presence of a signal peptide (cleaved site), Tat motif
Bacteria	β-*Proteobacteria*	*Parasutterella excrementihominis* YIT 11859	ZP_08323581.1	Flavocytochrome *c*	17	92	72/84	9×10^{-9}	57834.8	Tat peptide 50 amino acids [ANA-AA], NRRKFLS
	β-*Proteobacteria*	*Burkholderiales* bacterium 1_1_47	ZP_07343128.1	Flavin subunit of flavocytochrome *c*	6	88	71/79	3×10^{-8}	55660.9	Tat peptide 34 amino acids [ASA-NP], SRRNLLG
	β-*Proteobacteria*	*Sutterella wadsworthensis* 3_1_45B	ZP_08014924.1	Hypothetical protein HMPREF 9464_00143	15	88	92/92	2×10^{-8}	55183.1	Tat peptide 28 amino acids [ASA-KA], SRRKWLK
	γ-*Proteobacteria*	*Shewanella frigidimarina* NCIMB 400	YP_749210.1	Flavocytochrome *c*	2	92	72/84	9×10^{-9}	54852.5	Tat peptide 35 amino acids [ASA-KT], SRRHFLK
	γ-*Proteobacteria*	*Shewanella oneidensis* MR-1	NP_719167.1	Flavin subunit of flavocytochrome *c*	3	88	67/83	2×10^{-6}	53546.6	Sec peptide 21 amino acids [ANA-NT]
	γ-*Proteobacteria*	*Shewanella sediminis* HAW-EB3	YP_001475910.1	Flavin subunit of flavocytochrome *c*	3	92	56/72	4×10^{-6}	53684.7	Tat peptide 27 amino acids [VSA-NS], TRRSFLK
	δ-*Proteobacteria*	*Anaeromyxobacter dehalogenans* 2CP-1	YP_002492269.1	Flavocytochrome *c*	–	92	80/88	1×10^{-8}	55562.2	Tat peptide 38 amino acids [AEA-AE], GRRAMLK
	δ-*Proteobacteria*	*Anaeromyxobacter* sp. K	YP_002134140.1	Flavocytochrome *c*	–	92	80/88	1×10^{-8}	55546.2	Tat peptide 38 amino acids [AEA-AE], GRRAMLK
	δ-*Proteobacteria*	*Anaeromyxobacter dehalogenans* 2CP-C	YP_465303.1	Flavocytochrome *c*	–	92	76/84	5×10^{-8}	55669.4	Tat peptide 38 amino acids [ADA-AE], GRRAILK

Table. (Contd.)

Domain	Class	Organism	Sequence no. in the database	Protein, function	Additional similar sequences	Superposition area, % of the total fragment length	Identity/ similarity, % of the total fragment length	E	Calculated Mr, Da	Possible presence of a signal peptide (cleaved site), Tat motif
Bacteria	δ-*Proteobacteria*	*Geobacter lovleyi* SZ	YP_001951186.1	Flavocytochrome *c*	1	92	68/80	5×10^{-8}	56034.0	Tat peptide 43 amino acids [AEA-AC], TRRSFLK
	δ-*Proteobacteria*	*Desulfatibacillum alkenivorans* AK-01	YP_002429921.1	Flavocytochrome *c*	–	92	72/80	7×10^{-8}	54912.5	Tat peptide 42 amino acids [AQA-AA], KRRSVIK
	δ-*Proteobacteria*	*Desulfuromonas acetoxidans* DSM 684	ZP_01312786.1	Flavocytochrome *c*	1	85	70/78	7×10^{-7}	52627.5	–
	ε-*Proteobacteria*	*Sulfurospirillum deleyianum* DSM 6946	YP_003305131.1	Flavocytochrome *c*	2	96	65/73	1×10^{-7}	56038.5	Tat peptide 31 amino acids [AMA-AA], SRRDALK
	ε-*Proteobacteria*	*Campylobacter curvus* 525.92	YP_001408896.1	Flavin subunit of flavocytochrome *c*	2	92	64/72	2×10^{-6}	54984.4	Tat peptide 29 amino acids [TQA-AL], SRRNFVK
	ε-*Proteobacteria*	*Campylobacter concisus* 13826	YP_001465970.1	Protein with a FAD-binding domain	1	88	63/71	2×10^{-6}	55956.7	Tat peptide 31 amino acids [LMA-SP], SRRDFVK
	ε-*Proteobacteria*	*Campylobacter gracilis* RM3268	ZP_05624663.1	Flavin subunit of flavocytochrome *c*	3	88	58/71	4×10^{-6}	55265.3	Tat peptide 29 amino acids [ATA-AQ], SRRSFLK
	ε-*Proteobacteria*	*Campylobacter rectus* RM3267	ZP_03609186.1	Flavin subunit of flavocytochrome *c*	2	88	58/67	8×10^{-6}	55606.0	Tat peptide 31 amino acids [AQA-AV], SRRDFVK
	ε-*Proteobacteria*	*Wolinella succinogenes* DSM 1740	NP_906388.1	Flavin subunit of flavocytochrome *c*	–	92	52/68	8×10^{-6}	55920.0	Tat peptide 34 amino acids [ALA-EP], GRRDLIK
	Actinobacteria	*Slackia heliotrinireducens* DSM 20476	YP_003142730.1	Flavin subunit of succinate dehydrogenase/fumarate reductase	2	88	54/67	5×10^{-7}	63965.8	Sec peptide 22 amino acids [ESA-DG]

Table. (Contd.)

Domain	Class	Organism	Sequence no. in the database	Protein, function	Additional similar sequences	Superposition area, % of the total fragment length	Identity/ similarity, % of the total fragment length	E	Calculated Mr, Da	Possible presence of a signal peptide (cleaved site), Tat motif
Bacteria	*Actino-bacteria*	*Slackia exigua* ATCC 700122	ZP_06160356.1	Flavin subunit of a predicted fumarate reductase	2	96	46/54	8×10^{-7}	65961.5	Tat peptide 37 amino acids [AFA-TE], SRRNFLG
	Actino-bacteria	*Eggerthella* sp. 1_3_56FAA	ZP_07947477.1	Flavocytochrome *c*	8	88	58/67	4×10^{-6}	54766.9	Tat peptide 41 amino acids [ASA-AT], SRRSFIT
	Actino-bacteria	*Eggerthella* sp. HGA1	ZP_08164376.1	Flavocytochrome *c*	8	88	58/67	4×10^{-6}	55065.2	Tat peptide 44 amino acids [ASA-AT], SRRSFIT
	Actino-bacteria	*Eggerthella lenta* DSM 2243	YP_003183267.1	Flavocytochrome *c*	14	88	58/67	4×10^{-6}	55023.2	Tat peptide 44 amino acids [ASA-AT], SRRSFIT
	Actino-bacteria	*Gordonia neofelifaecis* NRRL B-59395	ZP_08206728.1	3-Ketosteroid-delta-1 dehydrogenase	–	92	44/60	4×10^{-6}	60114.2	–
	Actino-bacteria	*Frankia alni* ACN14a	YP_714564.1	Predicted succinate dehydrogenase	–	85	65/70	6×10^{-6}	58836.6	–
	Deferri-bacteres	*Denitrovibrio acetiphilus* DSM 12809	YP_003505239.1	Flavocytochrome *c*	2	92	80/88	3×10^{-8}	54982.4	Tat peptide 40 amino acids [AEA-AS], TRRGLLQ
	Clostridia	*Desulfitobacterium hafniense* Y51	YP_516518.1	Flavin subunit of succinate dehydrogenase/fumarate reductase	12	96	50/58	1×10^{-6}	55427.0	Tat peptide 39 amino acids [ASA-PE], NRRDFIK
	Clostridia	*Desulfitobacterium hafniense* DCB-2	YP_002458971.1	Predicted succinate dehydrogenase/fumarate reductase	8	96	58/65	3×10^{-6}	65706.1	Tat peptide 45 amino acids [QDA-QG], SRRDFLK
	Spirocha-etes	*Spirochaeta smaragdinae* DSM 11293	YP_003802577.1	Flavocytochrome *c*	–	92	60/72	3×10^{-6}	68957.7	Sec peptide 25 amino acids [GQA-SD]
Archaea	*Halobac-teria*	*Haloterrigena turkmenica* DSM 5511	YP_003405351.1	Predicted succinate dehydrogenase/fumarate reductase	–	85	57/65	3×10^{-6}	58458.0	–

(YP_002492269.1 and YP_002134140.1) of two *Anaeromyxobacter* species, differing in one amino acid, and of three flavocytochromes *c* (ZP_07947477.1, ZP_08164376.1, and YP_003183267.1) of the *Eggerthella* species (three to five amino acids). Two flavocytochromes *c* of bacteria *P. excrementihominis* YIT 11859 and *Burkholderiales* bacterium 1_1_47 (ZP_08324569.1 and ZP_07343128.1, respectively) differed only in length, the former being 4 residues shorter. Another pair of flavocytochromes of the same bacteria is of special interest: while the flavin subunit of the *Burkholderiales* fumarate reductase (ZP_07343174.1) is 26 N-terminal amino acids shorter than the *P. excrementihominis* YIT 11859 flavocytochrome *c* (ZP_08324449.1), the remaining parts of these proteins are identical. This finding confirms the signal character of the N-terminal sequence of the immature protein.

Comparison of the amino acid sequence of methacrylate reductase provided the information concerning the number of copies of similar sequences within the genomes of bacterial human pathogens (*Eggerthella, Parasutterella, Sutterella, Campylobacter*, and *Burkholderiales* bacterium 1_1_47) [19–21]. This conclusion was based on the data of the "Additional similar sequences" column in the table. For example, *P. excrementihominis* YIT 11859, apart from the flavocytochrome *c* (ZP_08323581.1), has 17 sequences similar to the N-terminal sequence of methacrylate reductase. *Sut. wadsworthensis* 3_1_45B contained 15 such sequences (apart from the hypothetical protein HMPREF 9464_00143), while *Eggerthella* species had 9–15 homologous fragments, and *Burkholderiales* bacterium 1_1_47 had 7 similar sites. *Campylobacter* species possessed 3–4 sites similar to the methacrylate reductase fragment. Although no data exist concerning the pathogenicity to humans of *Desulfitobacterium hafniense* strains dehalogenating chlorinated organic compounds, their genomes contain 9 (strain DCB-2) and 13 (strain Y51) homologous sequences. Members of the genera *Shewanella* and *Slackia*, as well as *Sulfurospirillum deleyianum* DSM 6946 and *Denitrovibrio acetiphilus* DSM 12809, which are also not known to be pathogenic, contain several (usually 3) copies of the homologous sequences similar to methacrylate reductase.

Among all *Geobacter* strains, only *G. lovleyi* SZ was found to possess the flavocytochrome *c* amino acid sequence (YP_001951186.1) exhibiting high similarity (80%) to the N-terminal end of methacrylate reductase. This is especially intriguing since the similarity between the 16S rRNA gene sequences of *G. sulfurreducens* AM-1 and *G. lovleyi* SZ is only 92% (Glaushko, unpublished data). The sequences similar to the methacrylate reductase sequence were not revealed in the genomes of more closely related *G. sulfurreducens* KN400 and *G. sulfurreducens* PCA.

The dendrogram demonstrating the similarity between the proteins selected for the table (mostly flavocytochromes *c*) is shown on Fig. 1. The phylogenetic tree of the relevant microorganisms based on their 16S rRNA gene sequences (Fig. 2) may be used to assess the phylogenetic relationship of the carriers of the proteins with homologous amino acid sequences. Fig. 1 shows that the homologous proteins form five clusters, two of which contain members of the *Deltaproteobacteria*. Among six members of this class, two organisms (*G. lovleyi* SZ and *Desulfuromonas acetoxidans* DSM 684) fall into the group together with *Shewanella* strains, while four others (three *Anaeromyxobacter* strains and *Desulfitobacillum alkenivorans*) fall into the group with *Spir. smaragdinae* and *Denitrovibrio acetiphilus.* Two groups were also found in the case of actinobacteria. For example, three *Eggerthella* strains were grouped together. According to the results of computer analysis, they possessed cleavable signal sequences (41–45 amino acids) with the same Tat motif SRRSFIT in the 9–12th position from the N-terminus. In another group of actinobacteria, two subgroups were found (Fig. 1 and table): in one of them, the homologous proteins of actinobacteria *F. alni* and *Gor. noefelifaecis* as well as that of the only *Archaea* member, *Haloterrigena turkmenica* DSM 5511, had no signal peptides, while in the second subgroup, PRED-TAT analysis revealed both the Sec peptide (*Slackia heliotrinireducens*) and the Tat peptide (*Slackia exigua*) for the homological proteins of *Slackia* strains.

Growth of the purple nonsulfur photosynthetic bacterium *Rhodopseudomonas palustris* Ac1 on acrylamide as a carbon source under anaerobic photoheterotrophic conditions was demonstrated in 2005 [22]. In this work, the authors suggested the similarity between the acrylate reductase of strain Ac1 and methacrylate reductase of *G. sulfurreducens* AM-1. Comparative analysis of amino acid sequences did not reveal, however, the similarity to any enzymes of *Rhodopseudomonas*.

DISCUSSION

Comparative analysis of the N-terminal end of methacrylate reductase of *G. sulfurreducens* AM-1 revealed the presence of similar sequences in the proteins (mainly flavocytochromes *c*) in the members of five phyla of the *Bacteria* domain and one phylum of the *Archaea* domain. The number of these proteins was much higher than was previously revealed [7, 8]. The highest similarity was found for the *Proteobacteria*, the most numerous phylum within the *Bacteria* domain. This group was named after Proteus, the ancient Greek shape-shifting god. This name reflects the diversity of the biochemical, physiological, and morphological characteristics within this group. The bacteria investigated

(1) are sulfur-reducers (*Campylobacter* spp., *Desulfuromonas acetoxidans, Geobacter lovleyi, Shewanella* spp., *Spirochaeta smaragdinae, Sulfuro-*

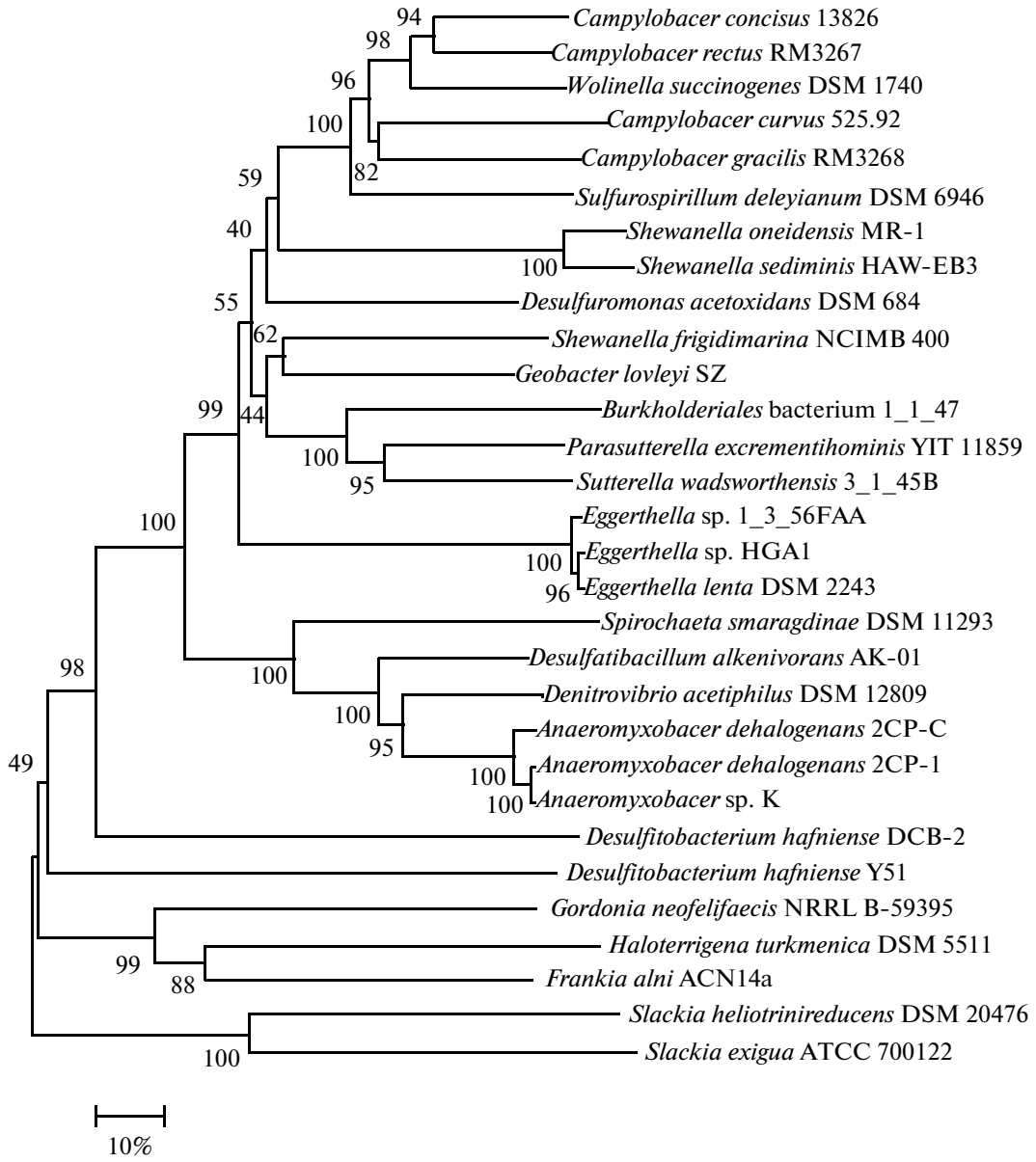

Fig. 1. Dendrogram showing relationships between the proteins exhibiting similarity to the N-terminal amino acid sequence of methacrylate reductase. Scale: 10 replacements per 100 positions.

spirillum deleyianum, Wolinella succinogenes) [23–25];

(2) oxidize acetate (*Anaeromyxobacter* spp., *Denitrovibrio acetiphilus, Desulfuromonas acetoxidans, Geobacter lovleyi*) [1, 23, 26];

(3) use fumarate as the terminal electron acceptor (*Campylobacter* spp., *Desulfitobacterium hafniense, Desulfuromonas acetoxidans, Geobacter lovleyi, Shewanella* spp., *Sulfurospirillum deleyianum, Wolinella succinogenes*) [7, 8, 27];

(4) reduce nitrate to ammonium (*Denitrovibrio acetiphilus, Sulfurospirillum deleyianum, Wolinella succinogenes*) [24, 28];

(5) transform chlorinated organic compounds (*Anaeromyxobacter* spp., *Burkholderiales* spp., *Desulfitobacterium hafniense, Geobacter lovleyi, Shewanella* spp.) [3, 4, 25, 27, 29];

(6) reduce metal oxides (*Anaeromyxobacter* spp., *Desulfitobacterium hafniense, Geobacter lovleyi, Shewanella* spp., *Sulfurospirillum deleyianum*) [1–3, 30];

(7) degrade hexahydro-1,3,5-trinitrotriazine, using it as a terminal electron acceptor (*Geobacter lovleyi, Shewanella* spp.) [31];

(8) contain high amounts of cytochromes *c* (*Anaeromyxobacter* spp., *Geobacter lovleyi, Desulfuromonas acetoxidans, Shewanella* spp.), which facilitate various

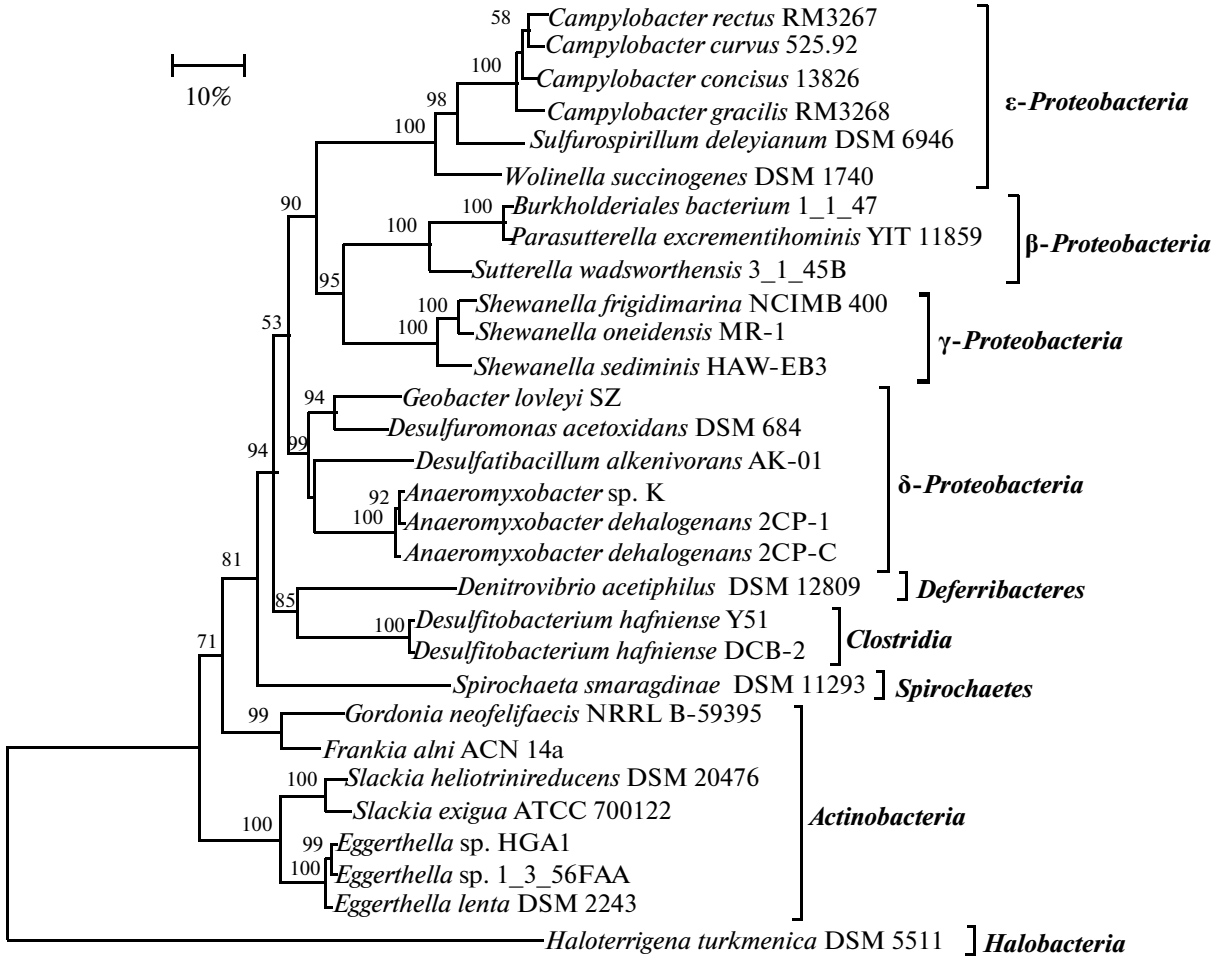

Fig. 2. Phylogenetic tree of the microorganisms possessing the genes for amino acid sequences homologous to the N-terminal end of methacrylate reductase. Scale: 10 replacements per 100 positions.

combinations of the reductase chains for growth on a broad spectrum of substrates [11–14, 23, 32];

(9) being mesophiles, grow at moderate temperatures (all).

Moreover, they cause diseases of the respiratory and digestive systems, as well as blood diseases (*Burkholderiales* sp., *Campylobacter* spp., *Eggerthella* spp, *Sutterella wadsworthensis*) [19–21]. Our results concerning the multiple copies of methacrylate reductase-like sequences in the genomes of these bacteria may be of interest for the understanding of pathogenesis and, as a consequence, promote recovery of the patients.

Pronounced homology of flavin-containing proteins is probably a result of their conservatism and early evolutionary origin. Most members of this family of proteins are found in bacteria capable of "anaerobic respiration" with a number of substrates (terminal acceptors of reduced equivalents) and possess the relevant redox systems. Periplasmic localization is suggested for most of the proteins revealed in the present work. The presence of methacrylate reductase-related proteins in phylogenetically remote organisms, as well as their presence in only some species within a genus, may result from significant horizontal gene transfer.

Relationships between the unique enzyme methacrylate reductase, which is able to reduce a non-natural substrate, and other proteins makes it possible to discuss two possibilities of its origin. The first one is that methacrylate reductase is a member of a numerous family of flavocytochromes *c* and flavoproteins possessing a number of reductase properties, including methacrylate reduction. The second one is that methacrylate reductase is a result of the evolution of flavocytochromes *c* and flavoproteins. Emergence of the enzyme with this substrate specificity in the course of natural mutagenesis results in an adaptive advantages for *G. sulfurreducens* AM-1 growing in microbial communities purifying the wastes of methacrylate industry.

These hypotheses are not mutually exclusive and are both supported by the research of the FccA protein in *W. succinogenes* [10]. In this work, the authors suggested the possibility of acrylate and methacrylate reduction by the periplasmic FccA flavoprotein or by

the complex of the FccA flavoprotein + the periplasmic FccB tetraheme cytochrome *c*. The N-terminal amino acid sequence of the *G. sulfurreducens* AM-1 methacrylate reductase exhibits 68% similarity to the flavoprotein FccA (table).

Unfortunately, apart from the periplasmic flavoprotein FccA (NP_906388.1) from *W. succinogenes*, none of the proteins with amino acid sequences similar to that of methacrylate reductase have been studied biochemically. The primary structure of these proteins was inferred from analysis of the complete genomes of the relevant bacteria. Thus, their physiological functions stated in the database are hypothetical. Reduction of methacrylate, which is a synthetic compound, is hardly the real physiological role of these flavoproteins in natural environments. However, the capacity of these proteins for methacrylate reduction is quite probable in anthropogenic environments such as wastewater treatment plants of plastic industry.

The results of the present work are of interest for the future investigation of the methacrylate redox system and of the relevant genes, providing for improved understanding of the mechanisms of double bond reduction by anaerobes. Knowledge of the molecular basis of anaerobic degradation of unsaturated toxic compounds will result in successful application of the proteins of the methacrylate reductase complex in environmental protection.

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