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EXPERIMENTAL ARTICLES

Investigation of Unusual Growth and Phenotypic Characteristics of Plasmid-Containing and Plasmid-Free Strains of Oligotrophic Bacterium *Ancylobacter vacuolatus*[1]

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Abstract—The oligotrophic bacterium *Ancylobacter vacuolatus* contains two large plasmids pREV1 and pREV2 (about 150 and 250 kb, respectively). Plasmid pREV1 carries the genes responsible for resistance to chloramphenicol, trimethoprim and γ-irradiation. Plasmid pREV2 carries the genes responsible for resistance to β-lactam antibiotics and formation of gas vacuoles. The ability to grow under oligotrophic conditions did not depend directly on either plasmid and is probably chromosome-encoded. Nevertheless, strains lacking the pREV2 plasmid had an improved capacity for growth in enriched media, as is evident from the following findings: 1) the growth rate of the strains lacking pREV2 was about 60% higher with an induction time of about two times less than those for strains carrying the plasmid; 2) the overall cell yield in rich media and colony size on non-oligotrophic agarized media increased with removal of pREV2; 3) the characteristic change in cell morphology occurring in the wild type of *A. vacuolatus* when switched from oligotrophic to eutrophic growth conditions was not observed in the strains lacking pREV2; 4) bacterial strains lacking pREV2 exhibited significantly higher rRNA content than the parent strain. As a possible explanation for these phenomena, we suggest that the pREV2 plasmid carries gene(s) for protein(s) acting as repressor(s) of expression of some enzymes involved in eutrophic metabolism. Such protein(s) probably participate in switching between the oligotrophic and eutrophic types of metabolism in response to changing nutrient supply in the environment.

Keywords: oligotrophy, large plasmids, metabolism, repression, *Ancylobacter*.

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Oligotrophic bacteria are a group of microorganisms capable of normal growth and reproduction on nutrient-deficient media. Their role in the soil microbial population is to utilize the residual monomeric nutrients, not consumed by hydrolytics (which mostly utilize polymers) and copiotrophs (which utilize monomers available in high concentrations) [1]. Oligotrophic bacteria are widespread and are usually the dominant group in natural ecosystems. They use the K-strategy [2, 3], constitute the most persistent part of the population, are highly competitive, and survive under stress conditions better than the other two groups [2]. Their ecological behavior may be defined as vigorous, due to their metabolic peculiarities, large pools of storage substances, and high trophic differentiation [1, 2, 4].

The present work was performed using an oligotrophic bacterium "*Renobacter vacuolatum*" [5] (=*Ancylobacter vacuolatus* DSMZ 1277 [6]) and was aimed at elucidation of the mechanisms responsible for the regulation of oligotrophic-type metabolism. According to the modern, mostly 16S rDNA-based classification, *A. vacuolatus* belongs to the class *Alphaproteobacteria*, order *Rhizobiales*, family *Xanthobacteraceae*. The presented experimental data allow us to suggest that at least some oligotrophs possess the genes required for eutrophic-type metabolism, most probably located on the chromosome. *A. vacuolatus* contains two large plasmids, one of which appears to encode a protein(s) acting as repressor(s) of some key pathways of eutrophic metabolism, i.e., affecting the enzymes with low affinity to the substrate. Such a protein(s) can play the role of a switch between oligotrophic and eutrophic metabolism when the nutrients concentration in the environment is changed.

MATERIALS AND METHODS

Bacterial strains. All bacteria used in this study were isolated from natural soil and water ecosystems and maintained in the laboratory collection. Characteristics of *A. vacuolatus* were described in [5]. The parent strain designated "strain W" forms snow-white colonies. "Strain G" was obtained as a colony which

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Table 1. Sensitivity of *A. vacuolatus* strains to selected antibiotics

Antibiotic	NP-300W	G	VZ-9	NP-854*
Actinomycin D, 30 μg	20 ± 2	16 ± 2	15 ± 4	31 ± 6
Doxycycline, 10 μg	10 ± 4	14 ± 2	21 ± 8	16 ± 2
Erythromycin, 15 μg	22 ±10	30 ± 6	28 ± 8	39 ± 5
Kanamycin, 30 μg	30 ± 6	35 ± 8	26 ± 5	29 ± 6
Neomycin, 30 μg	20 ± 3	22 ± 5	15 ± 3	27 ± 4
Novobiocin, 5 μg	16 ± 2	17 ± 5	10 ± 3	25 ± 3
Rifampicin, 5 μg	17 ± 2	17 ± 2	13 ± 3	24 ± 3
Streptomycin, 30 μg	38 ± 10	38 ± 8	29 ± 8	35 ± 5
Tetracycline, 30 μg	33 ± 10	36 ± 6	23 ± 6	14 ± 2
Ampicillin, 10 μg	–	19 ± 5	28 ± 6	–
Carbenicillin, 25 μg	–	20 ± 8	42 ± 15	–
Chloramphenicol, 30 μg	–	–	30 ± 3	45 ± 5
Puromycin, 30 μg	–	–	14 ± 2	30 ± 5
Trimethoprim, 5 μg	–	–	26 ± 5	NT
Others**	–	–	–	0

Notes. Data from three independent experiments are presented. The presented values are average sizes ± standard deviations (in mm) of growth-inhibition zones on agarized PYG medium. "–" no inhibition observed; NT – not tested.

* Control bacterium, *Hyphomonas* sp. NP-854.

** Other antibiotics: bacitracin, 30 μg; fusidic acid, sodium salt, 10 μg; lincomycin, 15 μg; methicillin, 10 mg; nitrofurazone, 50 mg; oleandomycin, 15 μg; polymyxin B, 30 u; ristomycin, 30 μg; rubomycin, 40 μg; sodium sulfacyl, 50 μg.

spontaneously lost the snow-white phenotype. It was shown to have only one plasmid (see Results).

To generate plasmid-free bacteria, cells from a single colony of strain G were treated with ethidium bromide [7]. After treatment, the cells were washed twice with the mineral medium (MM), containing the following (g/L tap water): KH_2PO_4, 1.36; $Na_2HPO_4 \cdot 12H_2O$, 3.3; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.005; $MnSO_4 \cdot 4H_2O$, 0.0025; $Na_2MoO_4 \cdot 2H_2O$, 0.0025; pH 7.0. Washed cells were plated on several petri dishes with agarized potato broth medium at the density of ~1000 cells/dish. The colonies were replicated with a velvet replica block onto several fresh agar plates supplemented with a selection of antibiotics. Clone VZ-9 lacking chloramphenicol resistance was chosen for further examination. It was confirmed to be plasmid-free (see Results).

Chemicals, media, and bacterial growth. The following media were used for bacterial growth: PYG (0.1% bacto-peptone, 0.1% yeast extract, and 0.1% glucose); 0.1× PYG and 5× PYG, 0.01%, and 0.5% of each of the above components, respectively; YP medium (0.01% yeast extract and 0.01% bacto-peptone supplemented with glucose at the required concentration); and potato-extract medium (PEM, ATCC medium 415). Agar was from Difco (BD, Franklin Lakes, United States). Sensitivity to antibiotics and heavy metals was determined by using antibiotics-, or metal-impregnated filter disks and PYG agar according to [8, 9]. Concentrations of the antibiotics and heavy metal salts are shown in Tables 1 and 2. Challenging bacteria with a second substrate (methanol) was performed according to [10]. Bacteria were grown in MM supplemented with 0.02% glucose to the early stationary phase, collected, resuspended in MM to equal density and transferred into MM with 0.1% methanol. Growth kinetics was followed by periodic measurements of turbidity in aseptically removed aliquots.

DNA isolation, separation, and sequence comparison; primers. Total DNA for PCR amplification and conventional agarose gel electrophoresis was isolated as described in [11]. DNA samples for pulse-field gel electrophoresis (PFGE) were prepared in 0.5% InCert agarose according to the manufacturer's recommendations for the CHEF Mapper XA system (Bio-Rad Laboratories, Hercules, United States). PFGE separation was carried out in 1% SeaKem agarose with 1/2 TBE buffer for 27 h using the system-built program for separation of DNA fragments within the range of 0.1–1 Mb. All types of agarose were from FMC Corp. (Philadelphia, United States). DNA sequence comparison was carried out using the BioEdit software package (Ibis Biosciences, Carlsbad, United States) and the Clustal X algorithm. The universal primers used were: pA (8F): AGAGTTTGATCCTGGCTCAG; pD (536R): GTATTACCGCGGCTGCTG; and pH (1542R): AAGGAGGTGATCCAGCCGCA

Table 2. Sensitivity of *A. vacuolatus* strains to selected cations and anions

Cation/Anion, concentration	NP-300W	G	VZ-9	NP-854*
Ag^{+}, 1 mM	14 ± 3	15 ± 3	16 ± 2	NT
Cd^{2+}, 1 mM	53 ± 11	44 ± 8	44 ± 8	20 ± 3
Co^{2+} 30 mM	55 ± 10	37 ±10	47 ± 8	16 ± 3
Cr^{3+}, 30 mM	32 ± 4	28 ± 4	29 ± 4	22 ± 2
Cr^{6+} ($Na_2Cr_2O_7$), 10 mM	50 ± 5	50 ± 4	50 ± 6	22 ± 5
Cu^{2+}, l0 mM	44 ± 10	46 ± 5	43 ± 6	11 ± 3
Fe^{3+}, 30 mM	53 ± 6	48 ± 5	48 ± 6	NT
Hg^{2+}, 1 mM	52 ± 10	46 ± 10	47 ± 8	13 ± 3
Ni^{2+}, 30 mM	31 ± 5	33 ± 4	32 ± 4	17 ± 3
Pb^{2+}, l0 mM	33 ± 6	25 ± 5	27 ± 5	–
Sn^{2+}, 10 mM	37 ± 6	38 ± 5	46 ± 6	NT
Zn^{2+}, 30 mM	40 ± 5	50 ± 5	45 ± 5	34 ± 4
Other**	–	–	–	NT

Notes. Data from three independent experiments are presented. The presented values are average sizes ± standard deviations (in mm) of growth-inhibition zones on agarized PYG medium. "–" no inhibition observed; NT – not tested.

* Control bacterium, *Hyphomonas* sp. NP-854.

** Other cations/anions used: As^{3+} ($NaAsO_2$ 1 mM); Au^{3+} (0.25 mM); F^{-} (1 mM); Mn^{7+} ($KMnO_4$ 10 mM); Mo^{6+} (Na_2MoO_4, 10 mM).

[11]. The sequence was deposited to GenBank (accession no. HM802213).

RESULTS AND DISCUSSION

Obtaining *A. vacuolatus* clones containing reduced numbers of plasmids. The oligotrophic bacterial strain *A. vacuolatus* NP-300 forms snow-white colonies on agar plates due to the presence of gas vacuoles [5]. Agarose gel-electrophoretic analysis of the total DNA isolated from these bacteria showed the presence of two large plasmids (pREV1 and pREV2), about 150 ± 10 and 250 ± 30 kb, respectively (size determined by PFGE) (Fig. 1). After several transfers in liquid media with subsequent plating on agar plates, a small percentage of colonies lost the characteristic white color and looked grayish (designated "strain G" as opposed to the parent "strain W"). Bacteria of this strain were morphologically identical to the parent strain. To confirm that grayish colonies did not result from contamination, we compared bacteria from the white and grayish colonies for their sensitivity to selected antibiotics and heavy metals and carried out 16S rRNA gene sequencing. Heavy metal and antibiotics sensitivity has been previously demonstrated to provide accurate taxonomic clustering for oligotrophic bacteria ([12] and Zlatkin, Vishnewetskaya, and Nikitin, unpublished data). The sensitivity pattern appeared to be virtually identical for both strains yet quite different from the oligotrophic bacterium *Hyphomonas* sp. NP-854 used as a control (Tables 1, 2). Sequence comparison (Fig. 2) showed that the partial 16S rRNA sequence obtained from clone G was virtually identical (with 3 nucleotide difference) to the published 16S rRNA gene sequence for *A. vacuolatus* NP-300 (reported as *Ancylobacter* sp. DSM-1277, accession number AY211515 [6]) and absolutely identical to the one from clone W. We believe the small differences with the published sequence may be the result of misreadings in the previous publication [6], because alignment including twelve more 16S rRNA gene sequences from related *Ancylobacter* species showed identity with our reads in all three questionable sites (data not shown). Thus, the grayish colonies belong to the species *A. vacuolatus*, as well as the snow-white ones. Significant difference between the two strains was revealed by agarose gel electrophoretic analysis of the total DNA which showed that strain G lacked the larger (pREV2) plasmid (Fig. 1).

To obtain a plasmid-free strain of *A. vacuolatus*, we treated strain G with ethidium bromide [7] with subsequent treatment of the replicas of the colonies with the antibiotics chloramphenicol, kanamycin, and tetracycline, to which strain G was resistant (the genes encoding resistance to these antibiotics are usually located on the plasmids, rather than on the chromosome). Using this approach, a bacterial colony which lost its ability to grow on the medium with chloramphenicol was isolated and designated *A. vacuolatus* strain VZ-9. Further analysis of this strain showed that, unlike strains W and G, it also lost resistance to trimethoprim and puromycin but otherwise responded to antibiotics and heavy metals identically to strain G (Tables 1a, 2), which confirms that it was not a con-

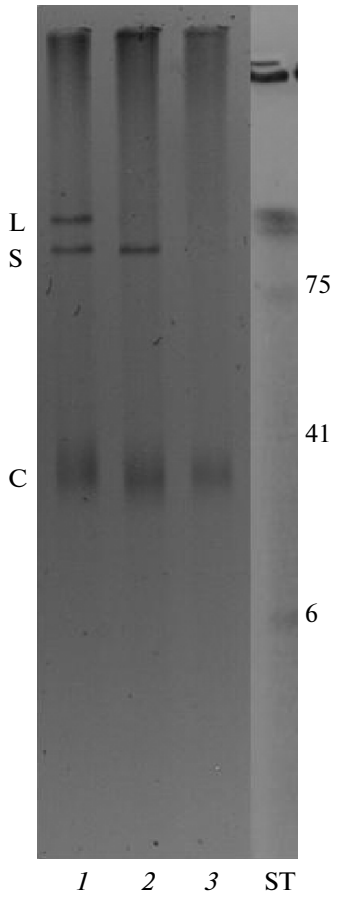

Fig. 1. Electrophoretic analysis of plasmid composition of *A. vacuolatus* strains. Agarose gel electrophoresis: *1*—wild type strain (W); *2*—the one-plasmid strain G; *3*—the plasmidless strain VZ-9 (L-larger and S-smaller plasmids; C-chromosomal DNA, ST-standard ladder: 75, 41, and 6 kB).

tamination. Plasmid analysis showed that strain VZ-9 was plasmid-free (Fig. 1).

Growth characteristics of *A. vacuolatus* strains. Comparative analysis of growth kinetics for all three *A. vacuolatus* strains (W, G, and VZ-9) showed that while they all grew similarly in low-carbon (oligotrophic) media, strain W grew significantly slower in rich liquid media and had the lag period much longer than the other two strains (Fig. 3, Table 3). Strains G and VZ-9 also formed larger colonies on rich agarized media (Table 4) than strain W. At the same time, the size of colonies was comparable for all three strains when grown on low-nutrient agarized media (Table 4). Noticeably, for strains G and VZ-9, morphology of growing cells did not change in enriched media, unlike strain W, which exhibited significant morphological changes when grown in enriched media ([13] and data not shown). When the cells from each of the three strains, after growth in MM medium with 0.02% glucose, were transferred to MM with 0.1% methanol, reaction of strain W to the second substrate was slower than that of either G or VZ-9 strains (Fig. 4).

Phenotypic characteristics of *A. vacuolatus* strains. All three strains were characterized by their sensitivity to a number of antibiotics (Table 1) and different heavy metals (Table 2), resistance to which is often associated with plasmid-encoded genes [14, 15]. It was found that strain G (containing only the plasmid pREV1) differed from the parent strain W (containing both pREV1 and pREV2) in the sensitivity to β-lactam antibiotics, while the plasmid-free strain VZ-9 became additionally sensitive to puromycin and chloramphenicol. No differences were found between the strains in resistance to inorganic compounds.

In addition, all three strains were characterized by their sensitivity to γ-irradiation. The parental strain W, as well as the one-plasmid strain G, were shown to be rather resistant to it, while strain VZ-9 was much more sensitive (Fig. 5).

Based on these results, we believe that the plasmid pREV1 contains the genes for resistance to chloramphenicol, trimethoprim, puromycin, and γ-irradiation, while the plasmid pREV2 harbors the genes for gas-vacuole formation and resistance to β-lactam antibiotics. It also harbors the gene(s) responsible for the differences in the growth patterns described in the previous section.

Effect of loss of the larger plasmid (pREV2) on the metabolic properties of the bacterium. Our previous phylogenetic analysis based on 5S rRNA sequences, membrane fatty acid and phospholipid composition, and antibiograms [8, 12, 16, 17] provided strong support to the idea that oligotrophic bacteria, though usually defined as an ecological group [18], are taxonomically distinct from eutrophic bacteria. According to modern taxonomy, they form several discrete groups belonging to the phylum *Bacteroidetes*, order *Sphingobacteriales*, family *Cytophagaceae* (*Arcicella*, *Flectobacillus*, *Spirosoma*, etc.) and the phylum *Alphaproteobac-*

Table 3. Growth rate (h^{-1}) of different strains of *A. vacuolatus* in some liquid media

Medium/strain and plasmids present in the strain	YP-medium, at glucose concentration of		PYG	PEM
	0.02%	0.2%		
W, pREVl, pREV2	0.040	0.090	0.153	0.195
G, pREVl	0.042	0.106	0.30	0.285
VZ-9, no plasmids	0.039	0.108	0.305	0.273

Note. The rates were calculated from the slopes of initial growth kinetics, Fig. 3.

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                10        20        30        40        50        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NP–300G  TGGCGGCAGGCTTAACACATGCAAGTCGAACGCCCCGCAAGGGGAGTGGCAGACGGGTGAGTAACACGTGGGGATCTGCCCAATGGTACGGAATAATTTC
DSM1277  gggcggcaggcttaacacatgcaagtcgaacgccccgcaaggggagtggcagacgggtgagtaacacgtggggatctgcccaatggtacggaataatttc
NP–300W  TGGCGGCAGGCTTAACACATGCAAGTCGAACGCCCCGCAAGGGGAGTGGCAGACGGGTGAGTAACACGTGGGGATCTGCCCAATGGTACGGAATAATTTC

               110       120       130       140       150       160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NP–300G  GGGAAACTGGGACTAATACCGTATGAGCCCGCAAGGGGAAAGATTTATCGCCATTGGATGAACCCGCGTCGGATTAGCTAGTTGGTGTGGTAAAGGCGCA
DSM1277  gggaaactgggactaataccgtatgagcccgcaaggggaaagatttatcgccattggatgaacccgcgtcggattagctagttggtgtggtaaaggcgca
NP–300W  GGGAAACTGGGACTAATACCGTATGAGCCCGCAAGGGGAAAGATTTATCGCCATTGGATGAACCCGCGTCGGATTAGCTAGTTGGTGTGGTAAAGGCGCA

               210       220       230       240       250       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NP–300G  CCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG
DSM1277  ccaaggcgacgatccgtagctggtctgagaggatgatcagccacactgggactgagacacggccca-actcctacgggaggcagcagtggggaatattgg
NP–300W  CCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG

               310       320       330       340       350       360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NP–300G  ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCGCCGACGAAGATAATGACGGTAGTCGGAGA
DSM1277  acaatgggcgcaagcctgatccagccatgccgcgtgagtgatgaaggccttagggttgtaaagctctttcgccgacgaagataatgacggtagtcggaga
NP–300W  ACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCGCCGACGAAGATAATGACGGTAGTCGGAGA

                410       420
                ....|....|....|
NP–300G  AGAAGCCCCGGCTAACTT
DSM1277  aaaagccccggctaactt
NP–300W  AGAAGCCCCGGCTAACTT
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Fig. 2. Alignment of the partial 16S rRNA gene sequence of the wild-type strain *A. vacuolatus* NP-300W, the one-plasmid derivative strain *A. vacuolatus* NP-300G, and the published 16S rRNA gene sequence of *A. vacuolatus* NP-300 (reported as *Ancylobacter* sp. DSM-1277, accession number AY21151). The three differing nucleotide positions are underlined.

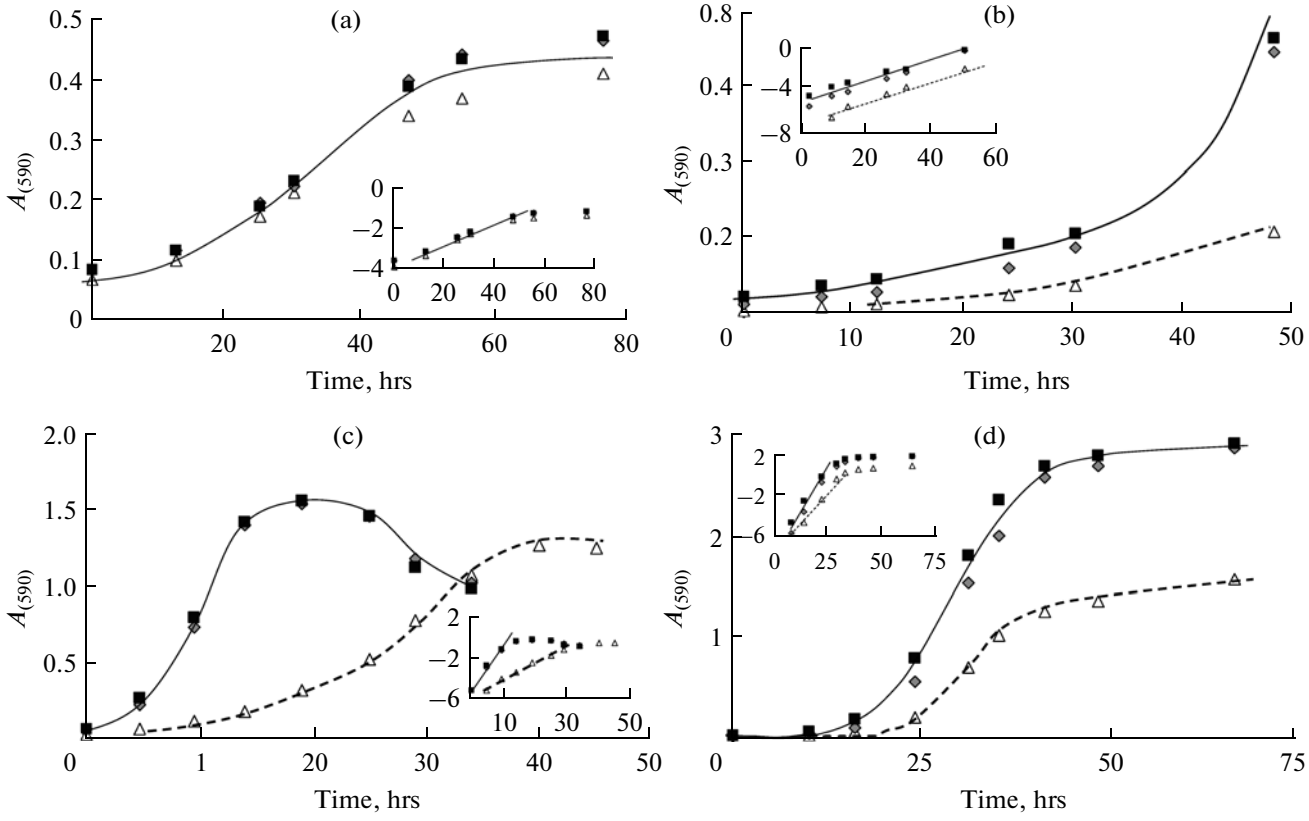

Fig. 3. Growth characteristics of *A. vacuolatus* strains in different liquid media. Note semi-log coordinates in the inserts. Open triangles—the wild-type strain W, shaded diamonds—the one-plasmid strain G, closed squares—the plasmidless strain VZ-9. The following media were used: YP with 0.02% glucose (a), YP with 0.2% glucose (b), PYG (c), PEM (d)0.

Table 4. Diameters of colonies (average ± standard deviation, cm) formed on agar plates by *A. vacuolatus* strains. From measurements of ten well-separated colonies

Medium/strain	YP-medium, at glucose concentration of		Low nutrition agar, 0.1× PYG	Medium nutrition agar, PYG	Rich nutrition agar, 5× PYG
	0.02%	0.2%			
W, pREV1, pREV2	1.87 ± 0.09	3.00 ± 0.07	0.60 ± 0.12	0.41 ± 0.1	0.85 ± 0.14
G, pREV1	1.93 ± 0.09	3.50 ± 0.44	0.40 ± 0.10	0.91 ± 0.1	1.79 ± 0.27
VZ-9 no plasmids	1.80 ± 0.13	3.70 ± 0.30	0.41 ± 0.10	0.94 ± 0.1	2.47 ± 0.21

teria, orders *Caulobacterales* (*Caulobacter*, *Hyphomonas*), and *Rhizobiales*, families *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Xanthobacteraceae*.

Although many explanations for the metabolic differences between eutrophs and oligotrophs have been suggested including bacterial "reticence" protecting the cells from excessive levels of nutrients [19], a combination of high specific affinity and low Michaelis constant [20], and $r \longrightarrow$ K transition in order to alleviate the toxic effect of reactive oxygen species [3], the phenomenon of oligotrophy is possibly based on specific molecular mechanisms, which are not understood.

Analysis of plasmid composition of several strains of oligotrophic *Alphaproteobacteria* from our laboratory collection showed that most of the obligate oligotrophic and only few of the facultative oligotrophic bacteria contained plasmids larger than 50 kb. This allowed us to suggest that large plasmids play an important role in obligate oligotrophy. Detailed study of this role was undertaken with the use of *A. vacuolatus* [6], an oligotroph forming snow-white colonies on agarized media due to the presence of gas vacuoles (GVF phenotype) and containing two large plasmids of approx. 150 and approx. 250 kb. Derivative strains containing only one or no plasmids were also created and analyzed.

Since plasmid-deficient strains retained the basic parent's properties while growing on/in low-nutrient (oligotrophic) media, it is obvious that their ability to grow under oligotrophic conditions is not plasmid-dependent and is most probably encoded by chromosomal genes. On the other hand, analysis of growth in/on nutrient-rich media showed that the loss of the larger plasmid pREV2 was associated with the acquisition of ability to grow faster and to higher cellular density. Altogether, strains G and VZ-9 exhibited very close growth characteristics on enriched media, which showed the following advantages over strain W:

1. The growth rate was about 60% higher (Table 3).
2. The lag phase duration was twice less (Fig. 3).
3. Overall cell yield of these strains was increased (Fig. 3).

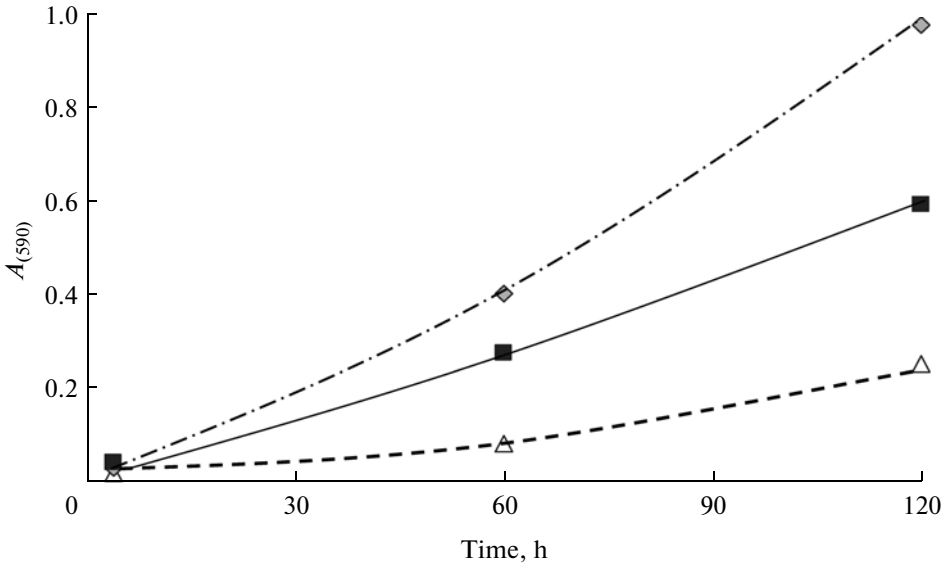

Fig. 4. Reaction of different strains of *A. vacuolatus* grown in glucose-containing medium to transfer into methanol-containing medium at time "0". Open triangles—the wild-type strain W, shaded diamonds—the one-plasmid strain G, closed squares—the plasmidless strain VZ-9.

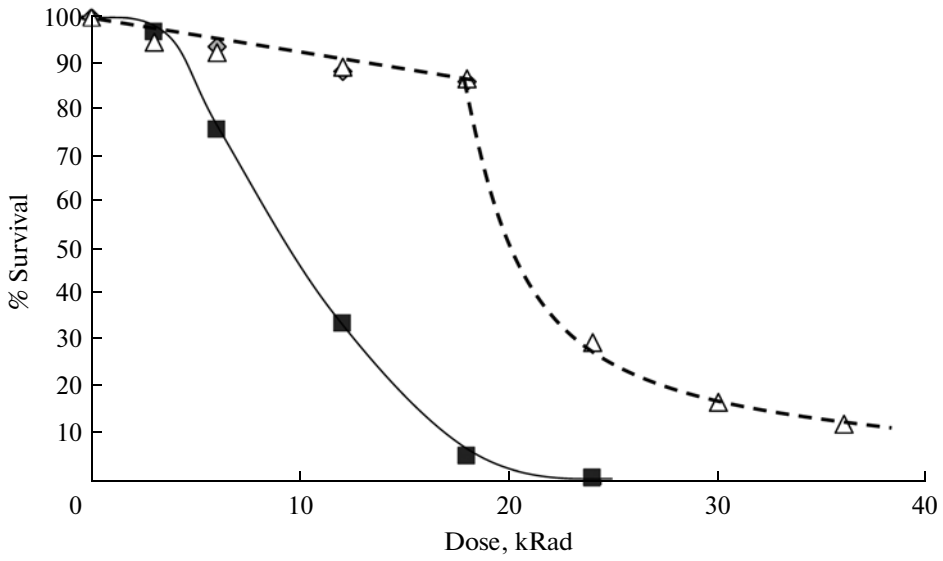

Fig. 5. Sensitivity of the *A. vacuolatus* strains to γ-irradiation. Open triangles—strain W, shaded diamonds—strain G, closed squares—strain VZ-9.

4. Their colony diameter on non-oligotrophic agar was increased (Table 4).

5. These strains showed higher capacity for utilization of the second substrate (Fig. 4).

Significant morphological changes of the cells growing in enriched media are a characteristic feature of obligate oligotrophic bacteria. However, changes in cell morphology described for strain W grown in rich media [13] were absent in the case of both plasmid-deficient strains (data not shown).

These data show that growth of *A. vacuolatus* in rich media (using eutrophic-type metabolism), while strongly inhibited in the case of the wild two-plasmid strain, was significantly better in the case of the plasmid-deficient strains. Therefore, strains G and VZ-9 lost the oligotrophic phenotype and acquired the characteristics of facultative oligotrophs. This change correlated with the loss of the larger plasmid pREV2.

Although improvement in growth characteristics associated with the loss of the pREV2 plasmid might be explained by the absence of the transcriptional/translational burden associated with the synthesis of gas-vacuole-forming proteins, and other (if any) plasmid-born proteins, this explanation is not supported by our experiments. Quantitative analysis of total rRNA content of the three strains (Fig. 6) showed that it was increased in plasmid-deficient strains ($W < G \leq VZ\text{-}9$). Since the level of expression of total rRNA generally reflects the level of the metabolic rate [21, 22], the experiment clearly demonstrated that the observed growth acceleration for plasmid-deficient strains was achieved via increase in the overall metabolic rate. As a possible explanation for these phenomena, we suggest that the pREV2 plasmid carries gene(s) encoding the protein(s) acting as repressor(s) of the key stages of carbon utilization. Such a protein could play a role in switching between oligotrophic-type and eutrophic-type metabolisms when the nutrient supply in the medium is changed.

The idea that a plasmid-encoded protein can participate in repression of bacterial metabolism is not entirely new. In his review, Chernin [23] hypothesized that large plasmids may control bacterial metabolism and distinguished two major types of plasmid interference into the chromosomal functions: switching to novel pathways and modification of already existing pathways. It is now generally accepted that large plasmids, carrying up to 30% of bacterial genetic information [24], encode important activities related to the adaptation of their host to specific and extreme environmental conditions and may have major impact on its metabolic function [25–29]. Large plasmids are abundant in bacteria from extreme environments, including oligotrophic ones. For example, *Alphaproteobacteria* from the deep-sea subsurface (where the conditions are extremely oligotrophic) often contained large plasmids [30]. Subterranean bacteria were also often found to contain very large plasmids (>150 kb), with bacteria from deeper sediments (generally more oligotrophic environments) containing larger plasmids more frequently than bacteria from the surface or a shallow subsurface environment [9]. Similar results were obtained for oligotrophic bacteria isolated from deep-subsurface Paguate sandstone of the Cerro Negro site in New Mexico, United States, where about 83% of the strains contained large plasmids (I.V. Zlatkin, unpublished data).

Additional support for the concept of the plasmid-encoded repressor is provided by the well-known phenomenon that primary bacterial isolates from

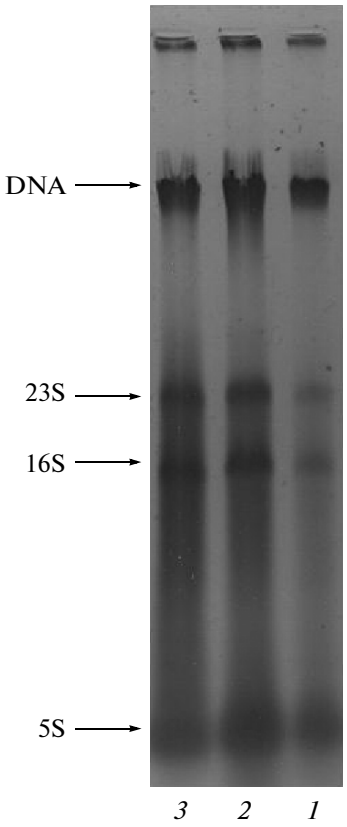

Fig. 6. Analysis of the total rRNA content in the cells of different strains of *A. vacuolatus* by gel electrophoresis in 1.5% agarose. *1*—wild type strain (W); *2*—the one-plasmid strain G; *3*—the plasmidless strain VZ-9. DNA denotes chromosomal DNA, and 23S, 16S, 5S denote 23S rRNA, 16S rRNA, and 5S rRNA, respectively.

extremely oligotrophic systems often cannot be grown in/on most artificial media upon initial isolation but can start growing on subsequent recultivation [31, 32]. A possible explanation for this phenomenon is that a single bacterial cell in the initial population has lost the large plasmid which was repressing its eutrophic metabolism, and thus gained the ability to grow in/on enriched media giving rise to the entire population.

In conclusion, the presented data allow us to suggest that: (1) oligotrophs possess the genes required for eutrophic-type metabolism; (2) the genes responsible for eutrophic metabolism are not plasmid-encoded and are therefore probably located on the bacterial chromosome; (3) large plasmids encode protein(s) acting as repressor(s) of some key pathways of eutrophic metabolism. Such a protein could play the role of a switch between oligotrophic and eutrophic metabolism when the nutrients' concentration in the media is changed.

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