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The RpoS Sigma Factor in the Dissimilatory Fe(III)-Reducing Bacterium *Geobacter sulfurreducens*

Cinthia Núñez,[1,2]* Lorrie Adams,[1] Susan Childers,[1,3] and Derek R. Lovley[1]

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003[1]; Department of Geological Sciences, University of Idaho, Moscow, Idaho 83844[3]; and Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México 62210[2]

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Geobacter sulfurreducens **RpoS sigma factor was shown to contribute to survival in stationary phase and upon oxygen exposure. Furthermore, a mutation in *rpoS* decreased the rate of reduction of insoluble Fe(III) but not of soluble forms of iron. This study suggests that RpoS plays a role in regulating metabolism of *Geobacter* under suboptimal conditions in subsurface environments.**

The physiology of microorganisms in the family *Geobacteraceae* in the δ-*Proteobacteria* is of special interest, because these are commonly the predominant organisms in sedimentary environments in which Fe(III) reduction is important (1, 14, 31, 32, 35, 36). Their ability to oxidize aromatic contaminants with the reduction of Fe(III) (24, 25), reductively precipitate uranium (1, 26), or grow via reductive dehalogenation (6, 16) suggests that they can contribute to the bioremediation of a variety of contaminants in subsurface environments, such as aromatic hydrocarbons (2, 31, 32, 35) and uranium (1, 14).

The *rpoS* gene encodes a subunit of the RNA polymerase which is the master regulator of the general stress response in *Escherichia coli* and related bacteria (8, 9). This response is observed when cells face a number of different adverse or suboptimal growth conditions and is commonly accompanied by a reduced growth rate or entry into stationary phase. Thus, in the *Geobacteraceae* RpoS might be expected to play a role in metabolic adaptation during growth in the subsurface, where it is expected to be more analogous to the stationary phase of culture than to the exponential phase. The goal of the present work was to initiate the study of the role of RpoS in *Geobacter sulfurreducens* in an attempt to understand the mechanisms of stress responses in the *Geobacteraceae*. *G. sulfurreducens* serves as a model because it is closely related to the *Geobacter* species that predominate in subsurface environments, it can be cultured in laboratory conditions, and its genome sequence (28) as well as a genetic system is available (5).

G. sulfurreducens **genome contains an *rpoS* homologue gene.** A search of the *G. sulfurreducens* genome sequence (www.tigr.org), using the *E. coli rpoS* gene as a query, revealed an open reading frame encoding a protein which has 42% identity with the RpoS protein of *E. coli* and consists of 328 amino acids with a molecular mass of 38 kDa. It has 87 and 68% amino acid identity with putative RpoS proteins of *Geobacter metallireducens* and *Desulfuromonas acetoxidans*, two other members of the *Geobacteraceae* (preliminary genome sequence data are available at http://www.jgi.doe.gov).

The genetic arrangement of the *G. sulfurreducens rpoS* region differs from that of *E. coli* and related bacteria. The *nlpD* gene, encoding an outer membrane lipoprotein (17), is absent, but this region contains a gene, downstream of *rpoS*, whose deduced amino acid sequence has 47.5% identity to the adenine phosphoribosyltransferase enzyme Apt of *E. coli*, which is involved in the one-step salvage pathway of adenine to AMP (12). Reverse transcription-PCR analysis demonstrated that *rpoS* forms part of the operon *surE-pcm-rpoS-apt* (data not shown). However, the presence of additional *rpoS*-containing transcripts cannot be discounted. Primer extension analyses of the *surE-pcm-rpoS-apt* operon were carried out, as described previously (3), with total RNA extracted from the wild-type strain DL1 (4) and *rpoS* mutant DLCN16(Δ*rpoS*::Km) grown in NBAF media (5) and with oligonucleotide surEPE (5′-GC GTGAACACCATCATC-3′) (which is complementary to the 5′ region of the *surE* gene). As shown in Fig. 1, the *surE*

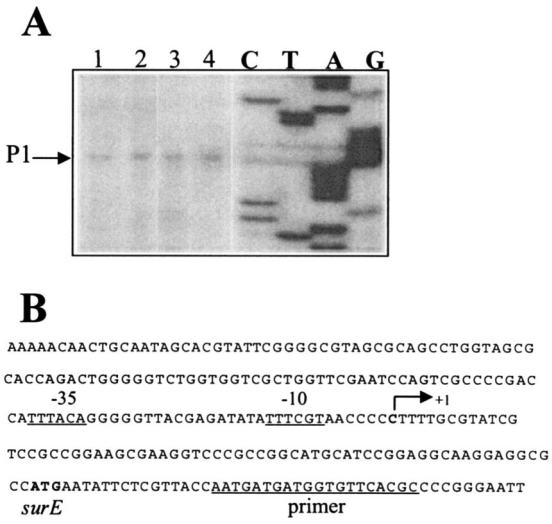

FIG. 1. Primer extension analysis of *surE* in *G. sulfurreducens* DL1 (lanes 1 and 3) and *rpoS* mutant DLCN16 (lanes 2 and 4). Total RNA was extracted from mid-log (lanes 1 and 2) or stationary-phase (lanes 3 and 4) cultures. The transcription initiation site P1 is indicated. (B) DNA sequence of the *surE* regulatory region. The arrow indicates the transcription start point.

* Corresponding author. Mailing address: Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa Cuernavaca, Morelos 62210, México. Phone: (52) 777 329-16-29. Fax: (52) 777 317-23-88. E-mail: cinthia@ibt.unam.mx.

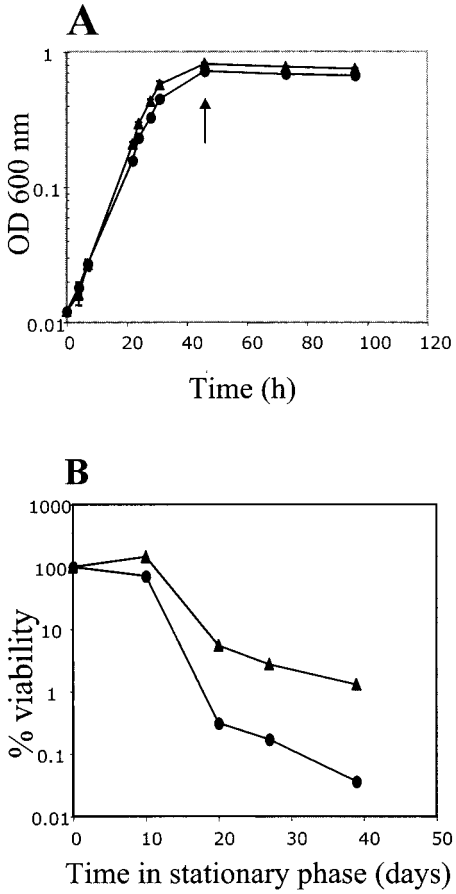

FIG. 2. (A) Growth of *G. sulfurreducens* DL1 (triangles) and *rpoS* mutant (circles) in media containing acetate as the electron donor and fumarate as the electron acceptor. Data are means of triplicates. The arrow indicates time zero of the survival curve. OD 600, optical density at 60 nm. (B) Stationary-phase survival of DL1 (triangles) and *rpoS* mutant DLCN16 (circles). Percent viability is expressed as the viable cell number at each time point divided by the viable cell number at time zero. Similar results were obtained in three experiments.

promoter contains −10 (TTTCGT) and −35 (TTTACA) sequences which are similar to the consensus sequences recognized by *E. coli* σ^{70}. This promoter was found to be RpoS independent either in the logarithmic or in the stationary phase of growth (Fig. 1). This is similar to *E. coli*, in which the transcription of *surE*, *pcm*, and *rpoS* is RpoS independent (10, 20). The *rpoS* mutant DLCN16(Δ*rpoS*::Km) was constructed as described previously (18), using the oligonucleotides rpoS1 (5′-CTTACATGGTCGCCCTGATG-3′) and rpoS2 (5′-CATGGAGATCTCCGTCGC-3′) to amplify the upstream region of *rpoS*, oligonucleotides rpoS5 (5′-CGAGGCCAAGT CTCTGG-3′) and rpoS6 (5′-GCCGTATTCGAGCTGATAG G-3′) to amplify the *rpoS* downstream region, and oligonucleotides rpoS3 (5′-GCGACGGAGATCTCCATGACCTGGG ATGAATGTCAGCTAC-3′) and rpoS4 (5′-CCAGAGACTTG GCCTCGAGAAGGCGGCGGTGGAATCG-3′) to amplify the kanamycin cassette. This cassette was inserted in the same orientation as that of *rpoS* transcription, resulting in a nonpolar mutation. This was confirmed by reverse transcription-PCR analysis using total RNA extracted from DLCN16 cultures and oligonucleotides designed to amplify the *apt* gene (data not shown).

Stationary-phase survival in the *rpoS* mutant, using NBAF medium with limiting acetate (8 mM) as electron donor and excess fumarate (37 mM) as electron acceptor (5), decreased at least 10-fold compared to that of the wild-type strain DL1, indicating that RpoS is involved in stationary-phase survival of *G. sulfurreducens* (Fig. 2). This result is consistent with the fact that *G. sulfurreducens rpoS* is cotranscribed with *surE*, *pcm*, and *apt*, whose products are necessary for stationary-phase survival in *E. coli* (7, 19, 38).

The utilization of either fumarate or soluble Fe(III) [in the form of Fe(III) citrate] as electron acceptor was not affected in the *rpoS* mutant (Fig. 2A and 3A); in contrast, the reduction of insoluble Fe(III) (Fig. 3B) was significantly diminished in the *rpoS* mutant compared to that of the wild-type strain. The same result was obtained with two independent *rpoS* mutant isolates. This result suggests that RpoS may regulate the expression of genes in *G. sulfurreducens* that are specifically required for the reduction of insoluble Fe(III) oxide, the primary

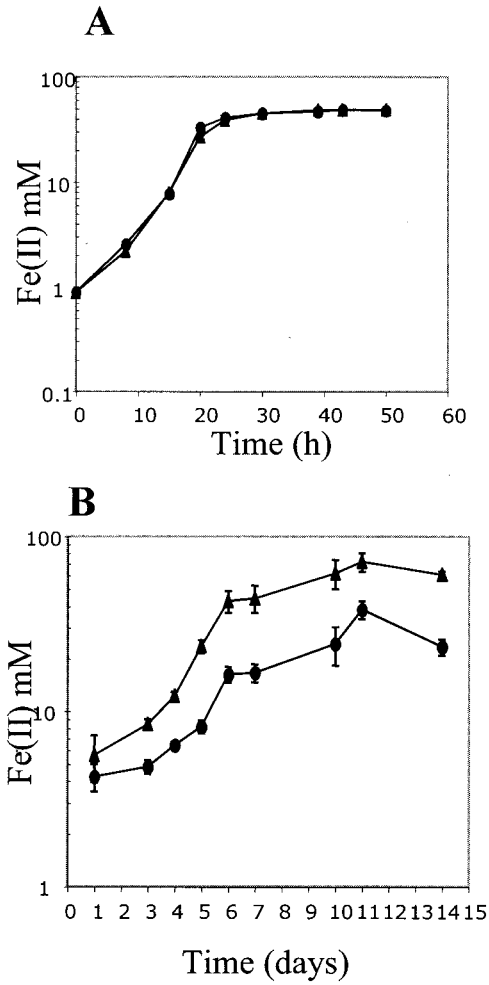

FIG. 3. Fe(III) citrate (A) or poorly crystalline Fe(III) oxide (B) reduction in the wild-type strain DL1 (triangles) and *rpoS* mutant (circles) in media containing acetate as electron donor. Determination of Fe(II) was carried out as previously described (30). Data are means of triplicates.

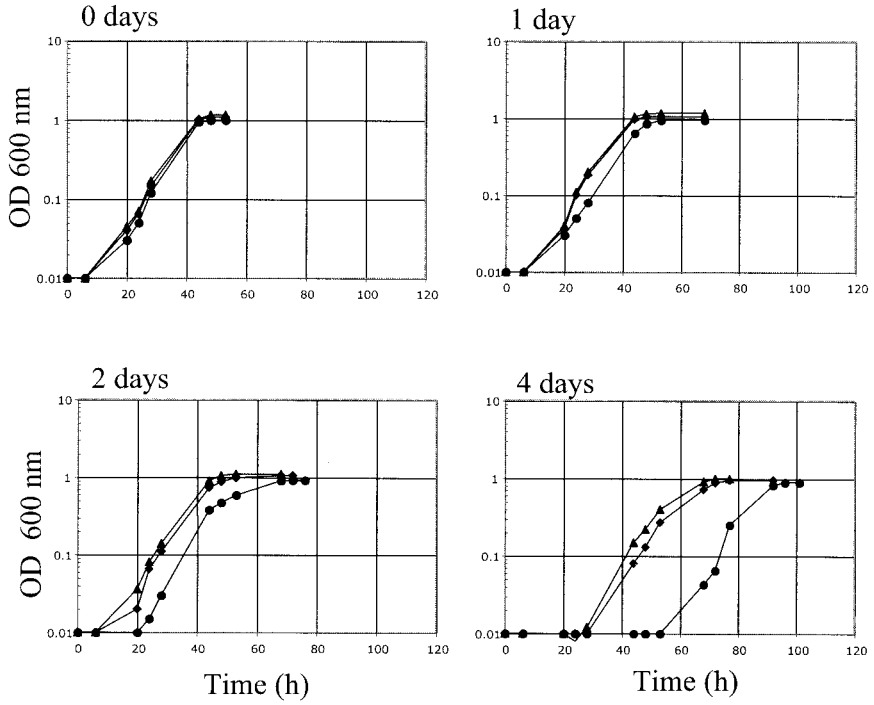

FIG. 4. Aerotolerance of DL1 (wild type) (triangles), DLCN46(*rpoS*::Cm) (circles), and DLCN52(*rpoS*::Cm/*rpoS*$^+$) (diamonds) after 0, 1, 2, and 4 days of air exposure. Data are means of triplicates. Survival was estimated as the lag time of growth of a culture derived from oxygen-exposed cells as described previously (21); determination of CFU was not feasible due to the fact that *G. sulfurreducens* forms cellular aggregates when exposed to oxygen, a trait that was enhanced by the *rpoS* mutation. OD 600, optical density at 600 nm.

form of Fe(III) in most sedimentary environments. Preliminary studies have indicated that the pattern of *c*-type cytochromes in the *rpoS* mutant is different from that in the wild-type strain (data not shown), which is significant because *c*-type cytochromes are involved in electron transfer to Fe(III) in *G. sulfurreducens* (18, 22, 27). The identity of such *c*-type cytochromes is presently being investigated.

Although *G. sulfurreducens* was originally designated a strict anaerobe (4), subsequent studies demonstrated that it can tolerate long-term exposure to oxygen (21). Oxygen intrusions into sediments in which *Geobacter* species predominate are common, and thus the ability to tolerate oxygen exposure is an important feature in the survival of these organisms (21). As shown in Fig. 4, tolerance to oxygen was clearly reduced in the *rpoS* mutant DLCN46(Δ*rpoS*::Cm) after 4 days of exposure and it was rescued in the *rpoS*-complemented mutant strain DLCN52(Δ*rpoS*::Cm/*rpoS*$^+$). (The procedure for the construction of a Δ*rpoS*::Cm mutant was the same as that for the DLCN16 mutant, except that oligonucleotides rpoSCm-3 [5′-GCGACGGAGATCTCCATGACGGAAGATCACTTCGC-3′] and rpoSCm4 [5′-CCAGAGACTTGGCCTCGAGGGCAGCAATAACTGCC-3′] were used to amplify the chloramphenicol cassette; the complementation was achieved, as described before [29], by cointegration of plasmid pCNDL17, a PCR 2.1-TOPO derivative [Km^r] [Invitrogen, Carlsbad, Calif.] carrying the entire *rpoS* gene.) These results indicate that RpoS is involved in the aerotolerance of *G. sulfurreducens*, which is consistent with its role in oxidative-stress resistance in other bacteria (15, 34, 37). However, the fact that the *rpoS* mutant of *G. sulfurreducens* was still viable after 4 days of oxygen exposure indicates that the mechanism for coping with oxidative damage is only partially RpoS dependent.

G. sulfurreducens RpoS had no apparent function in resistance to high temperature (45°C for 7 days) or alkaline pH (pH 6 for 60 min) (data not shown), indicating that some stress response mechanisms are not controlled by RpoS. In addition to RpoS and the well-known sigma factors RpoD (σ^{70}), RpoH (σ^{32}), RpoF (σ^{28}), and RpoN (σ^{54}), *G. sulfurreducens* contains a sigma factor that belongs to the family having extracytoplasmic functions (data not shown) (23). This sigma factor, designated RpoE, is likely to be involved in resistance to oxidative stress and other adverse conditions in *G. sulfurreducens* based on its role in *E. coli* and other gram-negative bacteria (11, 13, 33); however, this remains to be investigated.

This first study of RpoS in a member of the δ-*Proteobacteria* illustrates some similarities and differences in gene organization and function compared to those of other classes of previously investigated *Proteobacteria*. The effect of the *rpoS* mutant on survival in stationary phase and on reduction of Fe(III) oxide, the primary electron acceptor supporting the growth of *Geobacteraceae*, suggests that RpoS may play a role in controlling activity of *G. sulfurreducens* in subsurface environments. Thus, it seems likely that further investigation of the RpoS regulon will provide insights into the mechanisms by which *G. sulfurreducens* and related organisms function so effectively in Fe(III)-reducing subsurface environments.

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