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MINIREVIEW

Molecular Microbiology of the Oil Field Sulfur Cycle

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Oil fields harbor subsurface microbial communities that contain a rich diversity of bacteria. Because oil is produced by water injection as an oil-water mixture, the microbiologist has ready access to samples derived from these subsurface communities. The sulfate-reducing bacteria (SRB) have been studied most intensely, primarily because they are held responsible for souring (the increase in H_2S concentration in the produced oil with time) and anaerobic microbially influenced corrosion (MIC) of oil field equipment. Molecular biological studies have indicated that dissimilatory sulfite reductase, the enzyme producing the H_2S, is highly conserved in all SRB. The path used by SRB of the genus *Desulfovibrio* for electron transport from hydrogen to sulfate, thought to be similar to that traveled in MIC, has also been elucidated. Reverse sample genome probing (RSGP), a method developed to monitor multiple oil field bacteria in a single hybridization assay, has demonstrated that metal surfaces in oil fields are colonized by a limited number of SRB, which may be primarily responsible for MIC. These are resistant to the biocides used by field operators for their containment. The sulfide produced by SRB can be oxidized by endogenous nitrate-reducing, sulfide-oxidizing bacteria. One of these, *Thiomicrospira* sp. strain CVO, is able to oxidize sulfide to either sulfur or sulfate, while reducing nitrate to nitrogen. Large scale injection of nitrate into oil fields significantly reduces sulfide levels. Using RSGP, it was demonstrated that strain CVO became the dominant component of the microbial community, identifying it as the primary agent of souring reduction under these conditions. In conclusion, it appears that increased understanding of the microbiology of the oil field sulfur cycle at the molecular level is paving the way for improved oil recovery processes that are based on informed manipulation of the microbial community in oil fields.

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Molecular biology of Desulfovibrio

SRB of the genus *Desulfovibrio* are commonly found in soils, marine and fresh water sediments and in the subsurface, including in oil fields (5, 10). They are strict anaerobes that do not grow in the presence of air. However, they are equipped to survive prolonged periods of oxygen exposure in nature by the presence of oxygen-protection proteins such as superoxide dismutase, rubrerythrin and the recently described superoxide reductase (2). Oxygen exposure of a soil SRB, such as *D. vulgaris* Hildenborough (DvH) occurs when wet, anaerobic soil is subjected to long periods of dryness.

My laboratory has had a long-standing interest in developing the molecular biology of DvH. Because the genome of this organism is now being sequenced by The Institute of Genomic Research (TIGR) in Rockeville, MD, USA, we are focusing exclusively on mutagenesis of the DvH genome and analysis of the physiology of the resulting mutants. DvH uses mainly organic acids (e.g. lactate) or hydrogen as the electron donor for sulfate reduction. Hydrogen oxidation is now well understood at the molecular biological level. DvH has 3 hydrogenases, that catalyze the reaction $H_2 \leftrightarrow 2H^+ + 2e$. Two of these, the NiFe- and the NiFeSe-hydrogenase, contain nickel, whereas the Fe-only hydrogenase contains only Fe atoms in the active site. NiFe-hydrogenases are used widely by bacteria for hydrogen oxidation. However, periplasmic Fe-only hydrogenases are rare and are found only in the genus *Desulfovibrio*. Deletion of the genes for Fe-only hydrogenase does not prevent growth on media in which hydrogen is the sole electron donor for sulfate reduction, indicating that the NiFe-hydrogenase is possibly more important for hydrogen oxidation.

From periplasmic hydrogenase the electrons are thought to flow to periplasmic cytochrome c_3 and then through a transmembrane electron transport complex, the Hmc complex, to the sulfate reduction pathway, which is located in the cytoplasm (Fig. 1). The Hmc complex consists of 6 redox proteins, HmcA to HmcF (6). HmcA is the periplasmic high molecular weight cytochrome containing 16

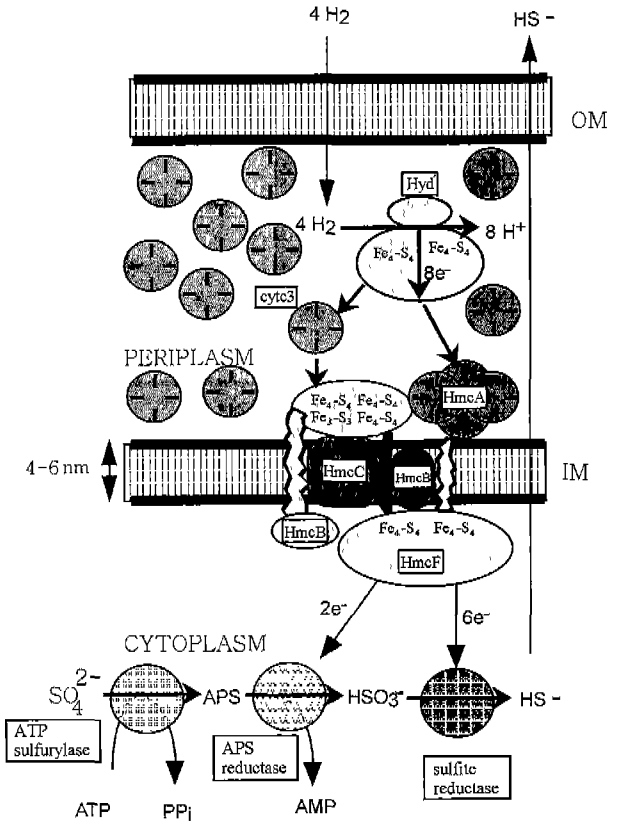

Fig. 1. Survey of redox proteins involved in the transfer of electrons from hydrogen to sulfate in *Desulfovibrio*. The inner membrane (IM) and outer membrane (OM) are indicated. The other terms are described in the text.

c-type hemes. HmcB to HmcE are integral membrane proteins, containing iron-sulfur clusters or *b*-type heme. HmcF is a cytoplasmic protein containing iron-sulfur clusters. All component proteins of the Hmc-complex are either membrane-bound or membrane-associated. The Hmc complex accepts electrons either from the periplasmic hydrogenases (e.g. from NiFe-hydrogenase) or from cytochrome c_3, transports them across the membrane and donates them to the cytoplasmic sulfate reduction pathway through HmcF. This has recently been proven by the generation of a mutant DvH H801 in which the genes for the Hmc complex have been deleted (1). The H801 strain is deficient in the use of hydrogen, but not in the use of lactate, as the electron donor for sulfate reduction. The cytoplasmic reduction of sulfate is catalyzed by 4 enzymes: ATP sulfurylase, adenosine-phosphosulfate (APS) reductase, dissimilatory sulfite reductase (Dsr) and pyrophosphatase (Fig. 1). APS reductase is highly conserved in SRB and this has formed the basis for an immunodetection assay for SRB in oil field environments (4). Dsr, an $\alpha_2\beta_2$ tetramer encoded by the *dsrA* and *dsrB* genes, is also highly conserved. At the protein sequence level the DsrA and DsrB sequences for the gram negative mesophilic eubacterium DvH and the thermophilic archaebacterium *Archaeoglobus fulgidus* are 60% identical (3). This has allowed the generation of PCR primers targeting conserved regions of the *dsr* genes, that can be used to demonstrate the potential for dissimilatory production of sulfide in the environment (3, 12).

Molecular biology of the oil field sulfur cycle

Oil fields harbor a variety of SRB. Some of these can use oil components as electron donors for sulfate reduction (7). Sulfide production by SRB is stimulated by water injection, especially when sea water, which contains 30 mM sulfate, is used. The gradual increase in sulfide concentration with time during oil production by water injection, is referred to as souring. Souring is considered a major problem by the industry because sulfide is toxic and corrosive. Reaction of sulfide with Fe^{2+} ions gives FeS, which contributes to the level of suspended solids in the injection water. High levels of suspended solids are considered undesirable by field operators, because they cause plugging near the well bore and thereby a loss of injectivity. In order to address these problems my laboratory has studied the diversity of SRB found in oil fields, particularly those in western Canada that are shallow (not deeper than 1000 m) and harbor a mesophilic population (30-40°C). For characterization of the diversity of SRB and other bacteria present in samples derived from oil fields we have developed reverse sample genome probing (RSGP) in which the whole genome of a microorganism is used as a probe for its detection in the environment (Fig. 2). RSGP analysis involves: (i) Culturing of a variety of bacteria (SRB, nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) and fermentative or heterotrophic bacteria) from the target environment. (ii) Analysis of the degree of cross-hybridization of the genomic DNAs obtained; only isolates with little or no genomic cross-hybridization are retained. These have been referred to as standards (11). (iii) Application of the derived minimal set of standard genomic DNAs to a filter (the master filter) in denatured form and in known concentrations (Fig. 2: 1-12). An internal standard (denatured bacteriophage λ DNA; Fig. 2: i1-i4) is also applied. The current master filter for analysis of the composition of mesophilic oil field communities contains the genomes for 30 SRB, 2 NR-SOB and 16 fermentative bacteria. Once a set of master filters (typically 200) has been made, analysis of the composition of environmental samples is straightforward (Fig. 2). With RSGP we have been able to directly analyze the microbial community that develops on metal parts of oil field pipelines and equipment. The reason for this interest is that SRB can use metallic iron [Fe^0] as electron donor for sulfate reduction and can thereby contribute to corrosion ($Fe^0 \rightarrow Fe^{2+} + 2e$). The sessile or biofilm communities, involved in MIC, were dominated by selected *Desulfovibrio* spp. With RSGP we could show that these appeared to be selected by the biocides used by the field

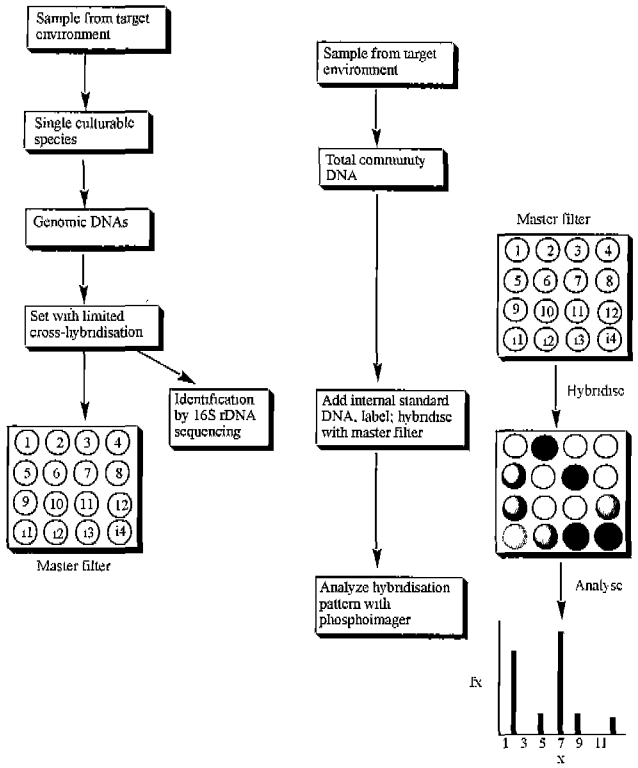

Fig. 2. Survey of the RSGP technique for analysis of the composition of microbial communities.

operators to prevent corrosion (8). RSGP has also been used successfully to monitor the effect of injection of nitrate into the Coleville field in Western Canada in order to remove sulfide. Two NR-SOB, *Thiomicrospira* strain CVO and *Arcobacter* strain FWKO B, had previously been isolated from this field. Strain CVO oxidizes sulfide with nitrate, forming nitrogen and either sulfur (S^0) or sulfate, depending on the sulfide to nitrate ratio, as the end products. Strain FWKO B oxidizes sulfide with nitrate with exclusive formation of S^0 and nitrite. Both of these organisms are autotrophs, using only CO_2 as the carbon source, and are novel oil field NR-SOB belonging to the ε-division of the proteobacteria. Addition of nitrate to waters injected into the Coleville field for a period of 50 days resulted in significant reduction (40-100%) of sulfide concentrations at both injection and producing wells for the duration of the nitrate application (9). RSGP analysis indicated that strain CVO became the dominant microbial community component under these conditions (Fig. 3). Following termination of nitrate injection the sulfide concentrations increased to values before nitrate injection was started and the fraction of strain CVO decreased to pre-injection levels. Thus reduction of souring by injection of nitrate can be credited to the action of strain CVO in this particular field. With RSGP we have demonstrated that strain CVO is also present in other shallow, low temperature fields in Western Canada, indicating that nitrate

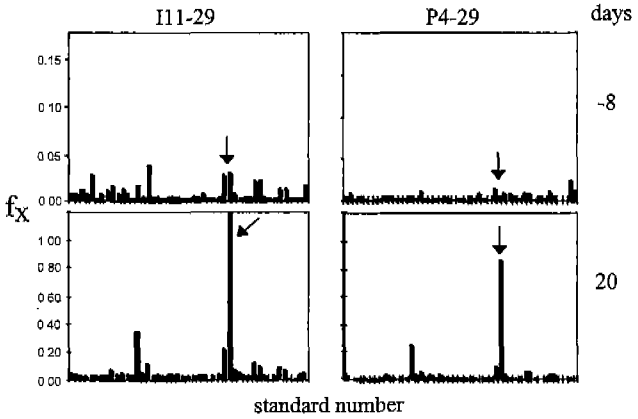

Fig. 3. Effect of addition of ammonium nitrate (0.5 g/l) to oil field injection water as monitored by RSGP. The calculated fraction f_x of each standard in the community is plotted for all standards on the master filter. The arrow indicates NR-SOB *Thiomicrospira* strain CVO. The community composition is shown before (8 days) and during (20 days) nitrate injection for injection well I11-29 and producing well P4-29.

injection may be a generally feasible strategy for reduction of sulfide concentrations.

Conclusions

Molecular biological study of SRB of the genus *Desulfovibrio* has progressed considerably in the last 15 years and will see even more rapid advancement with the expected completion of the sequence of the DvH genome. SRB are of great interest to the molecular microbiologist because they play a key role in the global, biological sulfur cycle. Understanding and managing this cycle in oil fields is bound to lead to economic spinoffs in terms of increased production of oil of better quality.

References

1. Dolla, A., B.K.J. Pohorelic, J.K. Voordouw, and G. Voordouw. 2000. Deletion of the *hmc*-operon of *Desulfovibrio vulgaris* subsp. *vulgaris* Hildenborough hampers hydrogen metabolism and low-redox-potential niche establishment. *Arch. Microbiol.* In press.
2. Jenney, Jr. F.E., M.F.J.M. Verhagen, X. Cui, and M.W.W. Adams. 1999. Anaerobic microbes: oxygen detoxification without superoxide dismutase. *Science* 286, 307-309.
3. Karkhoff-Schweizer, R.R., D.P.W. Huber, and G. Voordouw. 1995. Conservation of the genes for dissimilatory sulfite reductase from *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus* allows their detection by PCR. *Appl. Environ. Microbiol.* 61, 290-296.
4. Odom, J.M., K. Jessie, E. Knodel, and M. Emptage. 1991. Immunological cross-reactivities of adenosine-5'-phosphosulfate reductases from sulfate-reducing and sulfide-oxidizing bacteria. *Appl. Environ. Microbiol.* 57, 727-733.
5. Postgate, J.R. 1984. The sulphate-reducing bacteria, 2nd. Ed.

Cambridge University Press, Cambridge.
6. Rossi, M, W.B.R. Pollock, M.W. Reij, R.G. Keon, R. Fu, and G. Voordouw. 1993. The *hmc* operon of *Desulfovibrio vulgaris* subsp. *vulgaris* Hildenborough encodes a potential transmembrane redox protein complex. *J. Bacteriol.* 175, 4600-4711.
7. Rueter, P., R. Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.W. Jannasch, and F. Widdel. 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. *Nature* 372, 455-468.
8. Telang, A.J., S. Ebert, J.M. Foght, D.W.S. Westlake, and G. Voordouw. 1998. Effects of two diamine biocides on the microbial community from an oil field. *Can. J. Microbiol.* 44, 1060-1065.
9. Telang, A.J., S. Ebert, J.M. Foght, D.W.S. Westlake, G.E. Jenneman, D. Gevertz, and G. Voordouw. 1997. Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing. *Appl. Environ. Microbiol.* 63, 1785-1793.
10. Voordouw, G. 1995. The genus *Desulfovibrio*: The centennial. *Appl. Environ. Microbiol.* 61, 2813-2819.
11. Voordouw, G. 1998. Reverse sample genome probing of microbial community dynamics. *ASM News* 64, 627-633.
12. Wagner, M., A.J. Roger, J. L. Flax, G.A. Brusseau, and D.A. Stahl. 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol.* 180, 2975-82.