A genome probe survey of the microbial community in oil fields

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ABSTRACT

Oil fields of moderate temperature and salinity harbour a largely anaerobic microbial community, that includes a variety of sulfate-reducing (SRB), sulfide-oxidizing (SOB), and heterotrophic bacteria. Following culturing, these can be identified by 16S rRNA sequencing and distinguished by using their genomic DNA as a probe. A rapid assay for community composition is provided by the reverse sample genome probe (RSGP) method in which genomes from all isolated bacteria are spotted on a filter, which is then hybridized with extracted, labeled community DNA. With RSGP it has been shown that selected SRB are enhanced at metal surfaces, where they may contribute to corrosion, and that some of these SRB are rather insensitive to biocides that are used in the field. RSGP has also indicated that injection of nitrate, a higher potential electron acceptor than the commonly available sulfate, leads to a significant increase of a *Campylobacter* sp., which derives energy from the oxidation of sulfide by nitrate. These examples illustrate that RSGP can be used as a tool to monitor the effects of chemical stresses (biocides, nitrate) on the oil field microbial community, which may in turn help in the management of the direction of microbially-catalyzed processes in this environment.

Introduction

Oil is formed from biomass during burial and downward movement of sediments. Pressures and temperatures rise during this downward movement. Initial biomass components (lignin, carbohydrates, lipids, proteins) are converted to humic complexes and kerogen, and then to petroleum hydrocarbons and low molecular weight organic compounds [6]. Chemical reactions of organic acids (e.g. decarboxylations to hydrocarbons) are common in this latter phase. As a result the concentration of organic acids (e.g. acetic acid, propionic acid) in sedimentary basins decreases from ca. 5 g/L at 100^{o}C to 0.1 g/L at 160^{o}C. Below 100^{o}C the concentration of organic acids also drops sharply, but this is credited to microbial metabolism, not chemical decarboxylation [7].

Although oil forms at relatively high temperatures in the deep subsurface, its poor solubility in water and the high resident pressures cause lateral and upward migration until it is trapped under an impermeable sediment layer. The temperature in these oil-bearing zones may be much lower (20 to 50^{o}C at 300 to 900 m; 50 to 100 ^{o}C at 900 to 2000 m) than the oil formation temperature. When an oil-bearing zone is accessed by drilling, oil flows initially spontaneously to the surface driven by the high reservoir pressure (primary production). However, eventually this flow ceases and continued oil production requires water injection (secondary production). Water injection further decreases reservoir temperature and provides electron acceptors (e.g. sulfate, especially when sea water is used)

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (ed)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

for growth of a variety of mesophilic or thermophilic microorganisms depending on the resident temperature [4, 9, 11].

Growth of sulfate-reducing bacteria (SRB) on organic acids or hydrocarbons in the oil-bearing or surrounding sediments can lead to an increase in the sulfide concentration of the oil with time, a process referred to as souring. SRB can also use metallic iron as an electron donor for sulfate reduction [1, 2], causing anaerobic, microbially-influenced corrosion (MIC) of pipelines and oilfield equipment [3]. Souring and MIC are of concern to the industry because they lead to product and equipment failure, respectively. Control measures, e.g. the addition of biocides, are therefore frequently taken. In addition, there is interest in development of microbial means of sulfide removal in fields where sulfide concentrations are already substantial.

Results and Discussion

We have analyzed the microbial community in oil fields in western Canada (400 to 600 m, 20 to 40^{o}C) with reverse sample genome probing (RSGP) to identify bacteria responsible for MIC or souring control [10, 12]. Oil production in these fields is by water injection. The oil:water mixture is separated and the produced water is reinjected into the reservoir. In one of the fields studied two types of diamine biocides, A and B, were routinely added to produced waters to control microbial growth. In collaboration with Drs. D. W. S. Westlake, P. M. Fedorak and J. Foght of the University of Alberta in Edmonton (AB) and Dr. D. Gevertz of the Augouron Institute in La Jolla (CA) we have isolated 47 oil field bacteria that are genomically distinct. The genomic DNAs of these bacteria had little (generally less than 10%) crosshybridization when tested in dot blots under stringent hybridization conditions and we have referred to these bacteria as “standards” [10, 12]. This term is used to indicate that each standard may represent a set of closely related species with highly similar genomes. The 47 standards included 19 *Desulfovibrionaceae*, incompletely oxidizing SRB that can use organic acids as electron donor for sulfate reduction with production of acetate; 8 *Desulfobacteriaceae*, completely oxidizing SRB [13]; 1 anaerobic fermentative *Eubacterium* sp.; 1 nitrate-dependent sulfide oxidizer, CVO, a *Campylobacter* sp. [11] and 18 heterotrophs [10]. Most standards were identified by PCR amplification of 16S rRNA genes and comparing the sequence of the PCR product with a database of 16S rRNA sequences [10]. Known amounts (ca. 100 ng) of denatured genomic DNAs of these 47 standards were spotted on nylon membranes together with denatured bacteriophage λ DNA. The resulting master filters were hybridized with labeled community DNAs obtained or derived (following liquid enrichment culture) from oil field samples. Fractions f_x were calculated from these hybridization patterns for each standard without correction for crosshybridization [10].

In order to evaluate which of these 47 standards might contribute to MIC, we isolated total community DNAs from produced waters and from removable metal plugs that were installed and left in contact with these waters for 4 weeks. The resulting DNAs represented the planktonic and sessile communities, respectively, without culturing. The RSGP profiles of planktonic populations generally showed a more even distribution among the standards (Fig. 1A) than those of sessile populations (Fig. 1B). These were often dominated by selected *Desulfovibrionaceae*, e.g. in Fig. 1B the population is dominated by *Desulfovibrio* spp. Lac6 and Eth3. When produced water was used as inoculum for a liquid culture in

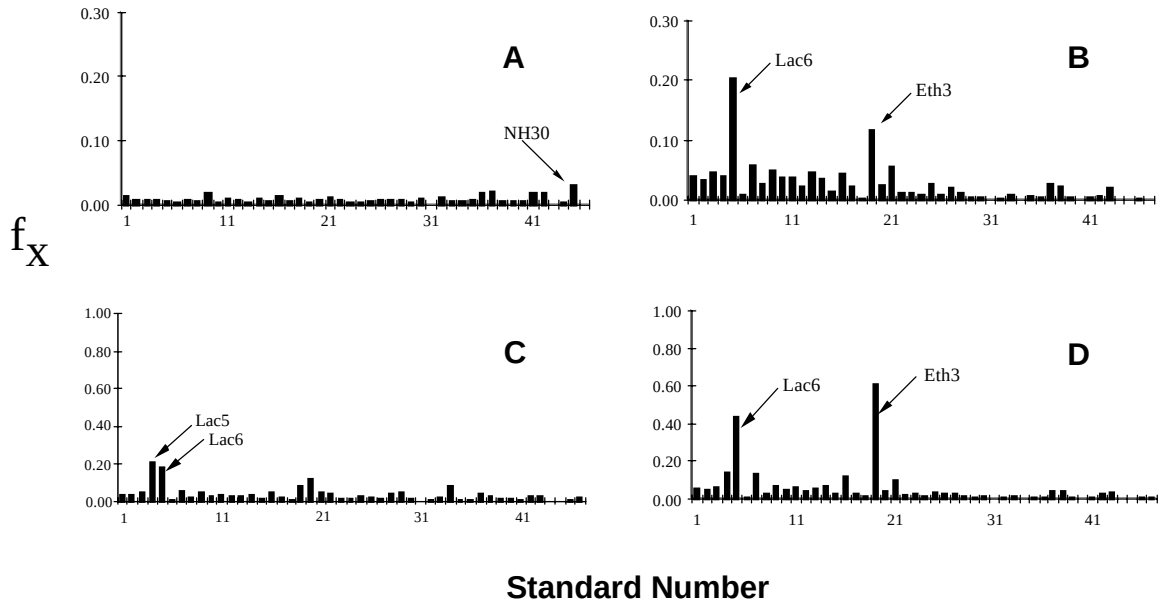

Fig. 1 RSGP profiles for the microbial community in an oil field. The fraction f_x calculated for each standard without correction for crosshybridization is plotted against standard number [10]. The profiles are for total community DNAs extracted directly from produced water (A), from an installed metal plug (B), from an enrichment culture of produced water in lactate-sulfate medium (C) and from an enrichment culture of produced water in lactate-sulfate medium to which 40 ppm of biocide B was added (D).

which lactate served as the electron donor for sulfate reduction the RSGP pattern in Fig. 1C was obtained. Addition of 40 ppm of biocide B shifted the pattern, which was now also dominated by *Desulfovibrionaceae* Lac6 and Eth3. Our interpretation of these results is first of all, that Lac6 and Eth3 grow better in a sessile biofilm than in a planktonic population (Figs. 1A and B, respectively). This indicates that these organisms derive energetic benefits from growth at a metal surface, relative to other members of the community. Secondly the emergence of these two organisms is also likely related to their biocide resistance (Fig. 1C and D, respectively). Lac6 and Eth3 were also resistant to 40 ppm A, 400 ppm B but not to 400 ppm A. Use of a mixture of organic acids (acetate, benzoate, lactate, propionate), instead of lactate alone, as electron donor for sulfate reduction gave the same result: only Lac6 and Eth3 were found to be significantly biocide resistant. Biocide concentrations of 400 ppm are close to the maximal dose recommended by manufacturers. Thus biocide B is ineffective in killing Lac6 and Eth3 under the chosen experimental conditions. Addition of these biocides is only effective in preventing MIC if the community present on the metal surface in the absence of biocides is more corrosive than the community (dominated by Lac6 and Eth3) selected in their presence.

A second application of RSGP has been to define the change in the oil field microbial community upon addition of nitrate to injection waters. In a field experiment ammonium nitrate was added to 400 mg/L for 50 days. The sulfide concentrations decreased by 40 to 100% following this addition. Most probable number assays indicated large increases in the number of nitrate-dependent sulfide oxidizers, while the numbers of lactate-utilizing SRB changed much less [10]. The changes in community structure associated with nitrate addition are shown in Fig. 2. Prior to nitrate addition CVO was a minor community

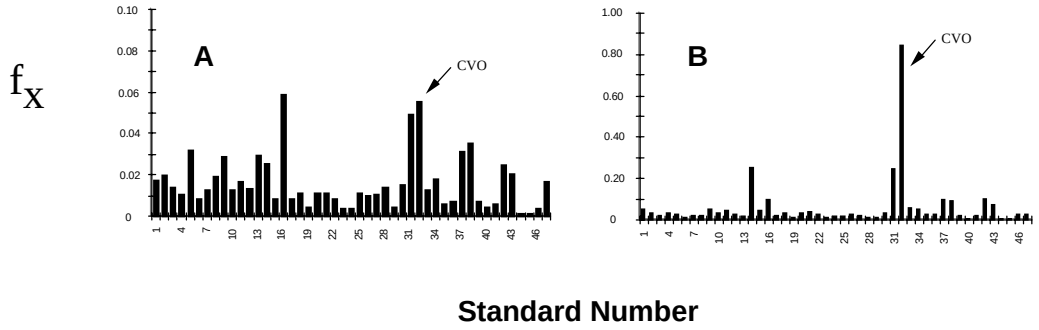

Fig. 2 RSGP profiles for an oil field microbial community in the absence (A) and presence (B) of 400 mg/L of ammonium nitrate. Nitrate was added in a field experiment for 50 days. Following cessation of nitrate addition the community reverted to the profile in (A).

component with a calculated $f_x = 0.05$, similar to other standards (Fig. 2A). However, following nitrate addition CVO became the dominant community component with a calculated $f_x = 0.9$ (Fig. 2B). CVO can derive energy for growth from the following redox reaction: $5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S^o + N_2 + 6\ H_2O$ [5]. Its dominance following addition of nitrate indicates that this higher potential electron acceptor is used by the oil field microbial community to reoxidize sulfide, not to oxidize hydrocarbon. The data in Fig. 2 indicate that large scale manipulation of a subsurface microbial community is feasible to achieve a desired goal (reduction in sulfide concentration).

In summary we have shown that RSGP is a useful tool to monitor the changes in an oil field microbial community when this is subjected to stresses (biocide, nitrate). The results help to interpret the nature of the microbial processes going on in this environment by identifying the microorganisms that are likely to be involved. Application of RSGP for describing the changes in environmental microbial communities, other than those in oil fields, are also feasible as reported recently [8].

Acknowledgments

This work has greatly benefitted from the microbiological expertise of Drs. D. W. S. Westlake, P. M. Fedorak, J. Foght and D. Gevertz and was funded through the NSERC Strategic Grants program.

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