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Identification of Distinct Communities of Sulfate-Reducing Bacteria in Oil Fields by Reverse Sample Genome Probing

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Thirty-five different standards of sulfate-reducing bacteria, identified by reverse sample genome probing and defined as bacteria with genomes showing little or no cross-hybridization, were in part characterized by Southern blotting, using 16S rRNA and hydrogenase gene probes. Samples from 56 sites in seven different western Canadian oil field locations were collected and enriched for sulfate-reducing bacteria by using different liquid media containing one of the following carbon sources: lactate, ethanol, benzoate, decanoate, propionate, or acetate. DNA was isolated from the enrichments and probed by reverse sample genome probing using master filters containing denatured chromosomal DNAs from the 35 sulfate-reducing bacterial standards. Statistical analysis of the microbial compositions at 44 of the 56 sites indicated the presence of two distinct communities of sulfate-reducing bacteria. The discriminating factor between the two communities was the salt concentration of the production waters, which were either fresh water or saline. Of 34 standards detected, 10 were unique to the fresh water and 18 were unique to the saline oil field environment, while only 6 organisms were cultured from both communities.

In a recent article, reverse sample genome probing (RSGP) was proposed as a novel DNA hybridization method for the identification of bacteria in environmental samples (17). The method allows a large number of bacterial standards to be analyzed simultaneously and is thus suitable for general screening of environmental samples. RSGP requires prior isolation of standards from the environment of interest and purification of their chromosomal DNA. The denatured standard DNAs are then spotted on a filter (the master filter) at a given concentration, which should ideally be the same for all standards. The sample DNA is then (i) denatured and spotted to provide a positive hybridization control and (ii) labeled and incubated with the master filter to identify qualitatively the standards prevailing in the sample. RSGP analysis of a limited number of oil field samples allowed the identification of 20 different sulfate-reducing bacteria (SRB) and gave a glimmer of the diversity of SRB populations in western Canadian oil fields (17). This diversity is more completely analyzed in the present paper.

MATERIALS AND METHODS

Biochemical reagents. Enzymes used for nick translation, primer extension, and 5′-end labeling were obtained from Pharmacia. Hybond-N hybridization transfer membrane was from Amersham. The radiolabeled compounds [α-^{32}P]dCTP and [γ-^{32}P]dATP (both 3,000 Ci/mmol, 10 mCi/ml) were from ICN, and [α-^{35}S]dATP (400 Ci/mmol, 10 mCi/ml) was from Amersham. Random hexadeoxyoligonucleotides were supplied by the DNA Synthesis Laboratory of The University of Calgary. Reagent-grade chemicals were purchased from either Fisher or Sigma.

Collection of samples. Samples were collected from six different oil fields and one oil storage facility in western Canada, as indicated in Fig. 1. At most fields oil was recovered by water flooding. The produced oil-water mixture was separated in a production unit, consisting of a free-water knockout and a water plant. Several production units were functioning in every field. The produced water was pumped back into the field, and the produced oil was pumped into the pipeline system. A different production unit was the truck pit, which received oil-water mixtures from a variety of field locations. Many of the collected samples represented these sites, as indicated in Table 1. Samples were taken either from the interior surface of a plug in a pipeline or directly from the fluid present at the site. These represented the sessile and planktonic microbial populations, respectively (Table 1). Samples were collected in sterile wide-mouth 500-ml plastic centrifuge bottles, which were filled to the top and used to inoculate serum bottles of prereduced media.

Liquid enrichment of SRB. Enrichment cultures of SRB were grown on media described by Pfennig et al. (13) with lactate, ethanol, benzoate, decanoate, propionate, or acetate as the carbon source at 22, 30, or 35°C. Usually 7.5 ml of the sample was used to inoculate a serum bottle with 75 ml of Pfennig's medium. The temperature chosen for incubation reflected that of the sample at the time of collection (Table 1). The salinity of the medium was likewise adjusted to reflect that of the sample (Table 1). One of three salinities was chosen: saline, 20.0 g of NaCl and 3.0 g of $MgCl_2$ per liter; brackish, 7.0 g of NaCl and 1.2 g of $MgCl_2$ per liter; and freshwater, 1.0 g of NaCl and 0.4 g of $MgCl_2$ per liter. Following growth the cultures were either used for isolation of DNA or stored at 22°C. Fresh medium (75 ml) of the same salinity was inoculated from these stored liquid cultures every month. Three classes of sample enrichments were distinguished, depending on the number of times (T) a sample was transferred: $T = 0$ represented the sample as obtained from the field, $T = 1$ was the primary enrichment, and $T > 1$ indicated subsequent enrichments (Table 2). No

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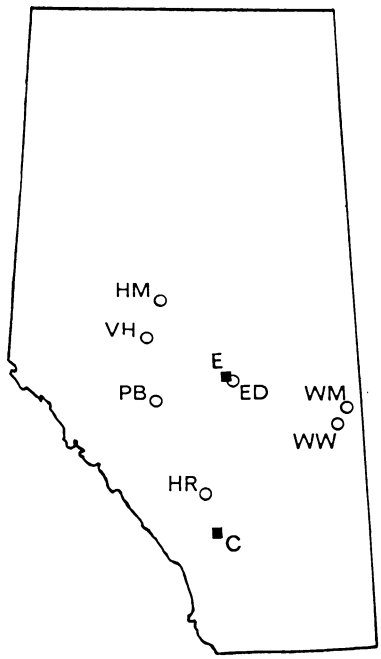

FIG. 1. Locations of six oil fields and an oil storage facility (ED) from which samples were collected in the province of Alberta, Canada. The following fields were sampled: House Mountain (HM), Virginia Hills (VH), Pembina (PB), Harmattan (HR), Wainwright (WW), and Wildmere (WM). The two main urban centers in Alberta, Calgary (C) and Edmonton (E), are indicated (separation, 277 km). The sites sampled at each of these seven locations are described in Table 1.

data from $T = 0$ enrichments are reported in the present paper. Liquid cultures of colony-purified isolates are also indicated (Table 2). Stable enrichment cultures, i.e., those yielding the same standard upon repeated transfers, were stored in glycerol at −70°C. As indicated previously (17), standards were named according to the carbon source used for their first isolation and an identifying number (Table 3).

Colony purification of SRB. All manipulations were carried out in a Coy anaerobic chamber (Coy Laboratory Products Inc., Ann Arbor, Mich.) in a mixed-gas atmosphere containing hydrogen, carbon dioxide, and nitrogen at 5, 10, and 85% (vol/vol), respectively. The carbon source was the same as that used for the generation of the enrichment culture throughout the entire procedure. Plates were incubated at 22 or 30°C. Selected liquid cultures were first streaked on Pfennig's medium plates containing 4% (wt/vol) agar. Colonies were picked after 4 to 6 weeks and used to inoculate culture tubes with 10 ml of modified Butlin's medium (4), containing (per liter) 0.5 g of K_2HPO_4, 1.0 g of NH_4Cl, 2.0 g of Na_2SO_4, 3 mg of $CaCl_2 \cdot 2H_2O$, 1.0 g of yeast extract, 4 mg of $FeSO_4 \cdot 7H_2O$, 10 ml of a 0.1% (wt/vol) solution of resazurin, and NaCl as described above for freshwater, brackish, or saline medium. Carbon sources were as in the media described by Pfennig et al. (13). Two iron nails were added to each culture tube, and the pH was adjusted to 7.5 prior to sterilization. The inoculated Butlin's tubes were incubated for 4 weeks to confirm the presence of SRB, which was indicated by blackening of the nails as a result of hydrogen sulfide production from sulfate. Cells from the blackened tubes were streaked onto plates of Pfennig's medium containing the appropriate carbon source, and after colonies appeared, these were transferred to Butlin's medium to verify that the isolates were SRB. This cycle was repeated until we were confident that pure cultures of SRB had been obtained, and then single colonies were used to inoculate Pfennig's medium. After growth cells were collected and either stored at −70°C in glycerol or used for DNA extraction.

DNA isolation and preparation of master filters. DNA was extracted and purified from cells either by a modified Marmur procedure (11), as described elsewhere (16, 17), or by a filter cartridge method to concentrate the cells of slowly growing cultures prior to DNA extraction (15). DNA preparations were dissolved in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8) at a concentration of 1 to 1,000 ng/μl, as determined by a fluorimetric procedure (17). A part of each preparation was then diluted to 10 ng/μl or used without dilution if the concentration was determined to be less than 10 ng/μl. Master filters were prepared by spotting 2-μl volumes of heat-denatured DNAs, prepared by placing 100 μl of DNA at 10 ng/μl in boiling water for 5 min followed by cooling on ice, for all standards in a known pattern on Hybond-N filter membrane. Denatured DNAs for up to 35 different standards (Table 3) were spotted with a 100-μl no. 710 Luer tip Hamilton syringe fitted with a pipette tip adapter and operated with a 1/50-volume dispenser. DNAs were covalently linked to the filters by irradiation with UV light (8,000 μW/cm^2, 312 nm) for 3 min.

DNA labeling for RSGP analysis. The denatured sample DNA preparation (2 μl, 10 ng/μl) was spotted on the master filter to provide a positive hybridization control (17). Reverse sample genome probes were prepared by primer extension for which 6 μl of denatured DNA solution, 6 μl of primer extension mix, 2 μl of Klenow polymerase (2 U/μl), 2 μl of [α-^{32}P]dCTP (3,000 Ci/mmol, 10 mCi/ml), and 14 μl of H_2O were combined in a microcentrifuge tube. Primer extension mix was made by combining 44 μl of 0.9 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) and 0.1 M $MgCl_2$ (pH 6.6), 25 μl of 1 M Tris-HCl (pH 7.4), 10 μl of 0.1 M dithiothreitol, 4 μl of 50 mM dGTP, 4 μl of 50 mM dATP, 4 μl of 50 mM dTTP, and 10 μl of 10 μg of random hexadeoxyoligonucleotides per μl. The reaction was conducted at 22°C for 3 to 5 h, after which the probes were boiled and added to the prehybridized master filters. Prehybridization, hybridization, washing, and autoradiography were done by the high-stringency procedure described before (16, 17).

Characterization of standards by Southern blotting. Chromosomal DNAs isolated from the SRB standards were digested with *Eco*RI and in some cases with *Hin*dIII. The digests were run on high-gelling-temperature (HGT) agarose gels and Southern blotted onto Hybond-N filter membranes as described elsewhere (16, 17). The blots were incubated with either of two probes. The 1.0-kb *hynBA* fragment, containing part of the genes for [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki, served as a probe for SRB from the genus *Desulfovibrio* (16), whereas a 16S rRNA probe isolated from *D. vulgaris* Hildenborough served as a general probe for the identification of all SRB standards. For preparation of the latter probe, *D. vulgaris* Hildenborough was cultured on 100 ml of Postgate's medium C (14). Total RNA was then extracted from the culture by the hot-phenol method (8) and precipitated with ethanol. Part of the precipitate was redissolved in TE and electrophoresed through HGT agarose. Following ethidium bromide staining, the 16S rRNA band was excised and the agarose was removed by boiling and three phenol extractions. The 16S rRNA was

TABLE 1. Survey of 56 sites from which samples were taken for SRB enrichment and subsequent RSGP analysis

Location[a]	Company	Site[b]	Description	Yr[c]	Type[d]	Sal[e]	Temp (°C)[f]	n[g]	SRB population[h]
Wainwright	A	WW1	Water plant 13	1989	p	s	30	4	*Lac4 Lac5 Lac10 Ben1*
Wainwright	A	WW2	Water plant 13	1989	s	s	30	3	*Lac5*
Wainwright	A	WW3	Water plant truck pit	1989	p	s	30	13	*Lac4 Lac5 Lac10 Ben1 Ben4 Dec3 Pro12*
Wainwright	A	WW4	Water plant	1989	s	s	30	5	*Lac4 Lac5 Lac10 Ben1 Ben4 Pro12*
Wainwright	A	WW5	Truck pit	1989	p	s	30	10	*Lac4 Lac5 Lac10 Ben1 Ben4 Dec3 Pro1 Pro12*
Wainwright	A	WW6	Water plant 1	1989	s	s	30	3	*Lac6 Eth3 Ben1*
Wainwright	A	WW7	Water plant 20	1989	p	s	30	7	*Lac4 Lac5 Lac10 Ben1 Pro1 Ace1*
Wainwright	A	WW8	Water plant 28	1989	s	s	30	4	*Lac4 Lac6 Lac10*
Wildmere	A	WM9	Wash tank	1989	s	s	50	0	No SRB recovered
Wildmere	A	WM10	Lower water plant	1989	s	s	35	12	*Lac4 Lac5 Lac10 Ben1 Dec8 Pro1*
Wainwright	A	WW11	Water plant 20	1990	p	s	30	5	*Lac4 Lac6 Eth3 Ben1 Dec1 Pro1*
Wainwright	A	WW12	Water plant 20	1990	s	s	30	6	*Lac6 Eth3 Ben1 Pro4 Ace1 Ace3*
Wainwright	A	WW13	Truck pit	1990	p	s	30	7	*Lac6 Pro4 Ace1 Ace3 Ace4*
Wainwright	A	WW14	Water plant truck pit	1990	p	s	30	7	*Lac6 Ben1 Pro4 Ace1 Ace4*
Wainwright	A	WW15	Water plant 13	1990	p	s	30	11	*Lac6 Ben1 Pro4 Ace1 Ace3*
Wainwright	A	WW16	Water plant 13	1990	s	s	30	7	*Lac6 Eth3 Ben1 Dec1 Dec3 Pro4*
Wainwright	A	WW17	Water plant 1	1990	p	s	30	8	*Lac4 Lac6 Lac12 Lac21 Eth3 Ben1 Ben3 Ace1 Ace3*
Wainwright	A	WW18	Water plant 28	1990	p	s	30	10	*Lac4 Lac6 Lac12 Lac21 Ben1 Dec8 Pro7 Pro11 Pro12*
Wainwright	A	WW19	Water plant 6	1990	p	s	30	6	*Lac6 Ben1 Dec1 Ace1 Ace3*
Wildmere	A	WM20	Lower water plant	1990	s	b	22	4	*Lac10 Ben1 Ben4*
Wildmere	A	WM21	Wash tank	1990	s	b	22	5	*Lac4 Lac5 Ben4 Dec1*
Wainwright	A	WW22	Water plant truck pit	1990	s	b	22	6	*Lac3 Lac6 Ben1 Pro1*
Wainwright	A	WW23	Wash tank truck pit	1990	s	b	22	6	*Lac3 Lac6 Ben1 Ben4 Dec1*
Wainwright	A	WW24	Water plant 20	1990	s	b	22	3	*Lac3 Lac6 Ben1 Ben4*
Edmonton	B	ED1	Storage tank 21	1989	p	f	22	3	*Lac8 Lac15*
Edmonton	B	ED2	Storage tank 22	1989	p	f	22	4	*Lac1,2 Lac8 Lac15*
Edmonton	B	ED3	Storage tank 23	1989	p	f	22	2	*Dec3*
Virginia Hills	C	VH1	Induced-gas flow inlet	1989	p	s	22	1	*Lac6*
Virginia Hills	C	VH2	Production water dump	1989	p	s	22	10	*Lac5 Lac6 Ben1 Dec1 Dec3*
Virginia Hills	C	VH3	Induced-gas flow outlet	1989	p	s	22	1	No confirmed identification
Virginia Hills	C	VH4	Free-water knockout	1990	p	s	50	0	No SRB recovered
Virginia Hills	C	VH5	Induced-gas flow outlet	1990	p	s	22	7	*Lac6 Lac21 Eth3 Ben1 Dec8 Ace1*
Virginia Hills	C	VH6	Sand filter outlet	1990	p	s	22	3	*Lac6 Lac12*
House Mountain	C	HM7	Unfiltered water	1989	p	s	22	6	*Lac6 Ben1 Dec3*
House Mountain	C	HM8	Pipe scrapings	1989	p	s	22	3	*Lac6 Ben1 Dec3 Ace4*
House Mountain	C	HM9	Unfiltered water	1989	p	s	22	3	*Lac6 Ben1 Dec3*
House Mountain	C	HM10	Produced well water	1990	p	s	22	8	*Lac6 Ben1 Dec1 Dec3 Pro4 Ace4*
House Mountain	C	HM11	Unfiltered combined water	1990	p	s	22	7	*Lac6 Ben1 Dec1 Dec3 Pro4 Pro12 Ace1*
House Mountain	C	HM12	Filtered combined water	1990	p	s	22	6	*Lac6 Dec3 Pro4*
Harmattan	C	HR13	Produced water	1988	p	b	35	13	*Lac3 Lac4 Lac10 Ben3 Ben4 Dec1 Pro4*
Harmattan	C	HR14	Flow splitter	1988	p	b	35	11	*Lac6 Lac12 Lac21 Eth2 Ben4*
Harmattan	C	HR15	Well 11-6	1988	p	b	35	5	*Lac4 Lac6 Lac12 Lac15 Lac21 Ben4*
Harmattan	C	HR16	Produced water	1990	p	b	35	1	No confirmed identification
Harmattan	C	HR17	Flow splitter	1990	p	b	35	3	*Lac21*
Pembina	D	PB1	Easyford battery	1990	p	f	22	8	*Lac12 Lac15 Pro5*
Pembina	D	PB2	Easyford battery	1990	p	f	22	6	*Lac12 Dec4 Pro5*
Pembina	D	PB3	Water two-stage separator	1990	p	f	22	6	*Lac12 Lac15 Pro5*
Pembina	D	PB4	Bear Lake, H battery	1990	p	f	22	10	*Lac3 Lac12 Lac15 Dec4 Pro5*
Pembina	D	PB5	NW Pembina B battery	1990	p	f	22	5	*Lac15 Pro5*
Pembina	D	PB6	Winfield A battery	1990	p	f	22	7	*Lac3 Lac7 Lac12*
Pembina	E	PB7	Injection water	1990	p	f	22	11	*Lac12 Lac15 Dec4 Pro10*
Pembina	E	PB8	8-2 skimmer	1990	p	f	22	11	*Lac12 Lac17 Eth2 Ben4 Ben6 Dec7 Pro10 Ace5*
Pembina	E	PB9	8-2 treater	1990	p	f	22	5	*Lac1,2 Lac12 Eth2 Ben6 Pro5 Pro10 Ace5*
Pembina	E	PB10	Injection well 6-1	1990	p	f	22	16	*Lac12 Lac15 Lac17 Ben4 Ben6 Dec6 Pro10 Ace5*
Pembina	E	PB11	Reservoir	1990	p	f	22	15	*Lac1,2 Lac12 Lac15 Lac21 Dec4 Pro5*
Pembina	E	PB12	16-7 produced water	1990	p	f	22	14	*Lac12 Lac15 Dec4 Dec6 Pro5 Ace5*

[a] Locations where samples were taken. All represent oil fields, except Edmonton, which represents an oil storage facility. See also Fig. 1.
[b] SRB populations at underlined sites are analyzed in Fig. 4; see also Table 2.
[c] Year in which the sample was collected.
[d] Sample type, either planktonic (p) or sessile (s); see text.
[e] Salinity used for cultivation: saline (s), brackish (b), or freshwater (f).
[f] Temperature used for cultivation of the sample.
[g] Number of RSGP assays performed to arrive at the SRB population.
[h] An SRB standard was considered part of the population only if it was found in at least two RSGP assays or if it was confirmed by conventional genome probing.

TABLE 2. RSGP identification results for 131 of the 367 DNA preparations analyzed

Prepn[a]	C source[b]	Status[c]	Date[d]	Identification[e]								
WW3												
1	Lactate	$T > 1$	90/2/28		*Lac5*							
2	Lactate	$T > 1$	90/11/28		*Lac5*							
3	Lactate	$T > 1$	91/3/27	*Lac4*	*Lac5*							
4	Lactate	CP	91/9/5		*Lac5*							
5	Lactate	CP	91/9/5		*Lac5*							
6	Lactate	CP	92/3/24		*Lac5*							
7	Lactate	CP	92/3/24		*Lac5*							
8	Ethanol	$T > 1$	90/3/20	*Lac4*	*Lac5*	*Lac10*	*Ben1*	*Ben4*				
9	Benzoate	$T > 1$	90/4/6				*Ben1*					
10	Decanoate	$T > 1$	90/5/16						*Dec3*			
11	Propionate	$T > 1$	91/3/27							*Pro12*		
12	Propionate	$T > 1$	91/5/13							*Pro12*		
13	Acetate	$T > 1$	90/5/31				*Ben1*					
WW4												
1	Lactate	T > 1	90/3/8	*Lac4*	*Lac5*		*Lac10*					
2	Ethanol	T > 1	90/3/15	*Lac4*	*Lac5*		*Lac10*	*Ben1*	*Ben4*			
3	Benzoate	T > 1	90/4/23					*Ben1*		*Pro12*		
4	Acetate	T > 1	90/5/31					*Ben1*				
5	Acetate	CP	91/11/6			*Lac6*						
WW5												
1	Lactate	$T > 1$	90/3/8		*Lac5*							
2	Ethanol	$T > 1$	90/3/15	*Lac4*	*Lac5*	*Lac10*	*Ben1*					*Pro12*
3	Benzoate	$T > 1$	90/4/23				*Ben1*					*Pro12*
4	Decanoate	$T > 1$	90/5/23							*Dec3*		
5	Propionate	$T > 1$	90/4/6								*Pro1*	
6	Propionate	$T > 1$	91/4/8						*Ben4*			
7	Propionate	$T > 1$	91/4						*Ben4*		*Pro1*	
8	Propionate	$T > 1$	91/6/14					*Ben3*	*Ben4*		*Pro1*	
9	Propionate	$T > 1$	92/1/29		*Lac5*							
10	Acetate	$T > 1$	90/5/24		*Lac5*		*Ben1*					
WM10												
1	Lactate	$T > 1$	90/4/10	*Lac4*	*Lac5*	*Lac10*						
2	Lactate	CP	91/9/5		*Lac5*							
3	Lactate	CP	92/3/24		*Lac5*							
4	Ethanol	$T > 1$	90/3/29		*Lac5*	*Lac10*						
5	Ethanol	$T > 1$	90/5/24		*Lac5*							
6	Benzoate	$T > 1$	90/5/24					*Ben1*				
7	Propionate	$T > 1$	90/4/20					*Ben1*				*Pro1*
8	Propionate	$T > 1$	91/5/13				*Eth2*			*Dec8*	*Dec7*	*Pro1*
9	Propionate	$T > 1$	91/6/14						*Ben4*			*Pro1*
10	Propionate	CP	92/1/29			*Lac10*						
11	Propionate	CP	92/1/29			*Lac10*						
12	Acetate	$T > 1$	90/5/31					*Ben1*				
WW15												
1	Lactate	$T > 1$	90/8/17								*Ace1*	*Ace3*
2	Ethanol	$T > 1$	90/10/3		*Eth3*	*Ben1*						
3	Benzoate	$T > 1$	90/8/24			*Ben1*						
4	Decanoate	$T > 1$	90/8/17					*Dec1*	*Dec3*		*Ace1*	*Ace3*
5	Propionate	$T > 1$	91/1/28							*Pro4*		
6	Propionate	$T > 1$	91/4							*Pro4*		
7	Propionate	$T > 1$	91/6/20			*Ben1*	*Ben4*					
8	Propionate	CP	92/1/29	*Lac6*								
9	Propionate	CP	92/1/29	*Lac6*								
10	Propionate	CP	92/1/29	*Lac6*						*Pro4*		
11	Acetate	$T > 1$	90/8/17								*Ace1*	*Ace3*
VH2												
1	Lactate	$T > 1$	90/3/21			*Lac6*						
2	Lactate	CP	91/9/5			*Lac6*						
3	Lactate	CP	92/3/24			*Lac6*						
4	Lactate	CP	92/3/24			*Lac6*						
5	Ethanol	$T > 1$	90/3/21	*Lac4*		*Lac6*	*Ben1*					
6	Benzoate	$T > 1$	90/4/20				*Ben1*					

Continued on following page

TABLE 2—*Continued*

Prepn[a]	C source[b]	Status[c]	Date[d]	Identification[e]								
7	Decanoate	$T > 1$	90/3/21					*Dec*1				
8	Decanoate	$T > 1$	91/4/12				*Ben*1	*Dec*1	*Dec*3			
9	Decanoate	CP	91/11/6		*Lac*5							
10	Decanoate	$T > 1$	92/1/29		*Lac*5							
HM7												
1	Lactate	$T > 1$	90/3/22	*Lac*4	*Lac*6							
2	Benzoate	$T > 1$	90/3/23			*Ben*1						
3	Benzoate	CP	92/1/6		*Lac*6							
4	Benzoate	CP	92/1/6		*Lac*6							
5	Decanoate	$T > 1$	90/4/17				*Dec*3					
6	Decanoate	CP	92/1/29		*Lac*6							
HR14												
1	Ethanol	$T > 1$	90/2/28		*Lac*6							
2	Lactate	$T > 1$	90/7/17		*Lac*6	*Lac*10						
3	Ethanol	$T > 1$	90/9/26							*Eth*2		
4	Ethanol	$T > 1$	91/1/28	*Lac*4			*Lac*12	*Lac*15	*Lac*21			
5	Ethanol	$T > 1$	91/4						*Lac*21			
6	Ethanol	$T > 1$	91/6/20				*Lac*12					
7	Ethanol	CP	91/11/6		*Lac*6							
8	Ethanol	$T > 1$	91/11/6		*Lac*6							
9	Benzoate	$T > 1$	90/12/13								*Ben*4	
10	Propionate	$T > 1$	90/12/12							*Eth*2	*Ben*4	
11	Acetate	$T > 1$	91/2/22								*Ben*3 *Ben*4	
PB7												
1	Lactate	$T > 1$	91/1/24				*Lac*15					
2	Lactate	CP	91/9/10				*Lac*15					
3	Lactate	CP	91/9/10	*Lac*1,2			*Lac*15					
4	Lactate	CP	92/2/13				*Lac*15					
5	Lactate	$T > 1$	92/3/24				*Lac*15					
6	Ethanol	$T > 1$	91/2/14		*Lac*7	*Lac*12	*Lac*15		*Dec*1			
7	Benzoate	$T > 1$	91/3/14				*Lac*15			*Dec*4		
8	Decanoate	$T > 1$	91/3/8							*Dec*4		
9	Propionate	$T > 1$	91/3/15			*Lac*12		*Lac*17				
10	Propionate	$T > 1$	91/5/17								*Pro*10	
11	Acetate	$T > 1$	91/2/14			*Lac*12				*Dec*4		
PB8												
1	Lactate	$T > 1$	90/11/28				*Lac*17				*Dec*7	*Pro*10
2	Lactate	$T = 1$	91/3/15	*Lac*1,2	*Lac*12		*Lac*17					
3	Ethanol	$T = 1$	91/3/15		*Lac*12		*Lac*17					
4	Ethanol	$T > 1$	91/4/12					*Eth*2		*Ben*6		*Pro*10 *Ace*5
5	Benzoate	$T = 1$	91/3/15							*Ben*6		
6	Benzoate	$T > 1$	92/1/29							*Ben*6		
7	Benzoate	$T > 1$	91/4/12					*Eth*2		*Ben*6		*Pro*10 *Ace*5
8	Decanoate	$T = 1$	91/3/15						*Ben*4		*Dec*7	*Pro*10
9	Propionate	$T = 1$	91/3/14				*Lac*17		*Ben*4			*Pro*10
10	Acetate	$T = 1$	91/3/15		*Lac*12		*Lac*17					
11	Acetate	$T = 1$	91/4/15			*Lac*15						
PB10												
1	Lactate	$T > 1$	91/1/24				*Lac*17					
2	Lactate	CP	91/10/8				*Lac*17					
3	Lactate	CP	91/9/10			*Lac*15	*Lac*17					
4	Lactate	CP	91/11				*Lac*17					
5	Lactate	CP	92/2/24			*Lac*15						
6	Lactate	$T = 1$	91/3/14				*Lac*17			*Ben*6		*Pro*10 *Ace*5
7	Ethanol	$T > 1$	91/2/14		*Lac*12							
8	Ethanol	$T = 1$	91/3/14				*Lac*17			*Ben*6		*Ace*5
9	Benzoate	$T > 1$	91/3/15	*Lac*4	*Lac*12	*Lac*15	*Lac*17		*Ben*4			
10	Benzoate	$T = 1$	91/3/14				*Lac*17			*Ben*6		*Ace*5
11	Decanoate	$T > 1$	91/3/8		*Lac*12	*Lac*15			*Ben*4		*Dec*6	
12	Propionate	$T = 1$	91/3/14		*Lac*12	*Lac*15	*Lac*17		*Ben*4			*Pro*10
13	Propionate	$T = 1$	91/3/14		*Lac*12		*Lac*17				*Dec*6	
14	Propionate	$T > 1$	91/5/17						*Ben*4		*Dec*6	
15	Acetate	$T = 1$	91/3/14				*Lac*17					
16	Acetate	$T > 1$	91/4/12					*Eth*2		*Ben*6		*Pro*10 *Ace*5

Continued on following page

TABLE 2—*Continued*

Prepn[a]	C source[b]	Status[c]	Date[d]	Identification[e]									
PB11													
1	Lactate	$T > 1$	91/1/24			*Lac*15							
2	Lactate	CP	91/10/8	*Lac*1,2									
3	Lactate	CP	91/10/8				*Lac*21						
4	Lactate	CP	91/10/8				*Lac*21						
5	Lactate	$T > 1$	91/11/4			*Lac*15							
6	Lactate	CP	92/1/30			*Lac*15							
7	Lactate	$T > 1$	92/3/24			*Lac*15							
8	Ethanol	$T > 1$	91/2/14		*Lac*12	*Lac*15							
9	Ethanol	$T = 1$	91/4/12					*Eth*2		*Ben*6			*Ace*5
10	Benzoate	$T > 1$	91/3/14			*Lac*15			*Ben*4		*Dec*4		
11	Decanoate	$T > 1$	91/3/8		*Lac*12							*Pro*5	
12	Propionate	$T > 1$	91/3/14									*Pro*5	
13	Propionate	$T > 1$	91/6/13									*Pro*5	
14	Propionate	$T = 1$	91/4/15									*Pro*5	
15	Acetate	$T > 1$	91/2/14		*Lac*12						*Dec*4		

[a] The site codes are as in Table 1, and an identifying number is assigned to each DNA preparation.
[b] DNA was prepared for RSGP analysis after enrichment or colony purification on the indicated carbon source.
[c] Status of the enrichment culture from which DNA was prepared, which was transferred from the sample either multiple times ($T > 1$) or only once ($T = 1$) or inoculated with a colony-purified isolate (CP).
[d] Date of DNA extraction and RSGP analysis (year/month/day).
[e] RSGP identifications made for the DNA preparations. Underlined identifications were confirmed by conventional genome probing of DNA preparations. The SRB populations derived from the RSGP data are given in Table 1.

then precipitated with ethanol and, after drying, dissolved in 50 μl of TE. The 16S rRNA was then treated with calf alkaline phosphatase and again phenol extracted and ethanol precipitated. The dephosphorylated 16S rRNA was labeled by reaction with [γ-^{32}P]ATP (3,000 Ci/mmol, 10 mCi/ml) catalyzed by T4 polynucleotide kinase. Southern blots were always incubated with either the 16S rRNA or the [NiFe] hydrogenase gene probe by using the low-stringency procedure in 50% (vol/vol) formamide at 42°C, as detailed elsewhere (16). Sizes of hybridizing bands were calculated relative to those of molecular size markers, prepared by digesting bacteriophage λ DNA with *Hin*dIII and 5′-end labeling with Klenow polymerase and [α-^{35}S]dATP.

Statistical analysis of bacterial population data. Derived populations were compared with SYSTAT (Systat Inc., Evanston, Ill.) run on an IBM-compatible personal computer.

RESULTS

Colony purification of SRB standards. Our success in colony purification of the SRB standards used in RSGP assays is summarized in Table 3. Of 35 standards that were spotted on the master filters in the present study, 2 were type cultures (*Lac*3 and *Ace*3 are *Desulfovibrio desulfuricans* G200 and *Desulfobacter hydrogenophilus*, respectively), 10 had been colony purified, and 14 represented stable enrichment cultures. Samples of these 26 standards were stored at −70°C in glycerol. Repeated transfer of enrichment cultures of nine standards resulted in the loss of the original standard from the enrichment, as analyzed by RSGP (Table 3). Confirmation of successful colony purification of standards *Lac*17 and *Lac*5 is shown in Fig. 2A and B.

Characterization of SRB standards by Southern blotting. Although the primary characteristic of all standards listed in Table 3 is that they have chromosomal DNAs that show little or no cross-hybridization in dot blots (e.g., see Fig. 2), it is desirable to characterize standards by a fingerprint procedure. *Eco*RI digests of chromosomal DNA from all standards were therefore analyzed by agarose gel electrophoresis and Southern blotting (10, 16). The blots were incubated with either the [NiFe] hydrogenase gene probe or a 16S rRNA gene probe. Use of the *Desulfovibrio*-specific hydrogenase gene probe has been described before (16). Specific hybridization was observed for 14 standards, 6 standards gave results that were inconclusive (either because no digestion was obtained or because different DNA preparations obtained for the standard gave different hybridization patterns), and 15 standards did not hybridize with the probe (Table 3). Contrary to the dot blot results reported in a previous paper (17), standard *Ben*1 did not hybridize with the hydrogenase probe, as judged from Southern blot results obtained for DNAs from 18 different enrichment cultures. Standards *Pro*1 and *Dec*1 were previously found not to hybridize in a dot blot procedure (17).

Fingerprint characterization of the 15 standards that did not react with the hydrogenase probe required the more general 16S rRNA probe. All SRB standard DNAs hybridized with this probe, although some showed only weak hybridization (Fig. 3).

Identification of SRB in oil field samples by RSGP. Each sample collected from the 56 sites listed in Table 1 was used to inoculate six serum bottles with Pfennig's medium containing one of the carbon sources: lactate, ethanol, benzoate, decanoate, propionate, or acetate. Thus, following growth and DNA extraction, 336 DNA preparations theoretically would be obtained for RSGP analysis. The number of preparations actually analyzed was influenced by failure of some of the samples to grow on some or all of the media provided and by the fact that some enrichment cultures (e.g., WW3 grown on lactate [Table 2]) were analyzed repeatedly. Also, the RSGP results for colony-purified isolates obtained from a sample have been entered in Table 2. Thus, a variable number of DNA preparations (0 to 16) was eventually obtained for each of the 56 collected samples. A total of 367 DNA preparations was analyzed by RSGP, and some of the results obtained are presented in Table 2. The RSGP identification for DNA preparations PB8-8 and WW5-8 is shown in Fig. 2C and D. Only clearly positive signals were considered and entered as SRB standards identified in Table 2. For

TABLE 3. SRB standards, isolated from oil field samples, that were initially identified by RSGP

Standard[a]	Carbon source	Status[b]	Size(s) (kbp) of fragment(s)[c] hybridizing with:	
			16S rRNA probe	[NiFe] hydrogenase probe
*Lac*1[d]	Lactate	CP	9.8, 7.5, 6.7, 5.5, 4.4, 2.1, 1.5	4.7, 2.6, 1.0
*Lac*2[d]	Lactate	CP	13.4, 11.3, 10.1, 8.1, 7.0, 5.9, 2.5, 1.2	4.7, 2.4, 1.0
*Lac*3	Lactate	TC	>23, 21.0, 17.2, 8.4, 5.5, 1.7	5.2, 0.36
*Lac*4	Lactate	CP	>23, 11.9, 2.8	12.1
*Lac*5	Lactate	CP	>23, 10.2, 6.6, 3.3	11.9
*Lac*6	Lactate	CP	4.3, 2.1, 0.9, 0.8	5.2
*Lac*7	Lactate	CP	9.8, 5.0, 4.0, 3.2, 3.0	No hybridization
*Lac*8	Lactate	—	11.0, 9.3, 6.6	14.1
*Lac*10	Lactate	CP	3.9, 3.5, 3.1, 2.0, 0.9	8.4
*Lac*12	Lactate	LC	10.9, 5.5, 5.3, 4.8	5.0, 1.0
*Lac*15	Lactate	CP	5.9, 4.7, 4.3, 3.8, 1.7, 0.9	1.9, 1.0
*Lac*17	Lactate	CP	4.4, 3.7, 3.3, 2.1, 1.8, 1.3	2.9
*Lac*21	Lactate	CP	3.5	Not conclusive
*Eth*2	Ethanol	—	Not conclusive	Not conclusive
*Eth*3	Ethanol	LC	3.2	3.9
*Ben*1	Benzoate	LC	Not conclusive	No hybridization
*Ben*3	Benzoate	—	Not conclusive	No hybridization
*Ben*4	Benzoate	—	8.3, 1.5	No hybridization
*Ben*6	Benzoate	—	Not conclusive	No hybridization
*Dec*1	Decanoate	LC	4.4	8.3
*Dec*3	Decanoate	LC	15.5, 8.5, 7.2, 5.2	No hybridization
*Dec*4	Decanoate	LC	Not conclusive	No hybridization
*Dec*6	Decanoate	LC	Not conclusive	Not conclusive
*Dec*7	Decanoate	—	Not conclusive	No hybridization
*Dec*8	Decanoate	—	Not conclusive	Not conclusive
*Pro*1	Propionate	LC	8.3	10.2
*Pro*4	Propionate	CP	8.9, 7.3, 6.5	No hybridization
*Pro*5	Propionate	LC	5.1	No hybridization
*Pro*7	Propionate	—	Not conclusive	No hybridization
*Pro*10	Propionate	LC	1.8	Not conclusive
*Pro*11	Propionate	LC	5.5	4.1
*Pro*12	Propionate	LC	7.5, 5.8, 3.4	No hybridization
*Ace*1	Acetate	LC	10.3, 5.8	No hybridization
*Ace*3	Acetate	TC	4.8, 4.4, 3.0	No hybridization
*Ace*4	Acetate	—	11.4, 7.4, 5.4	No hybridization
*Ace*5	Acetate	LC	Not conclusive	Not conclusive

[a] Standards are named by the first three letters of the carbon source used for their first isolation and an identifying number. Standards with the same name as in an earlier publication (17) are identical to those in that publication.

[b] CP, single colony purified in this work or a previous work (17); TC, type culture; LC, stable enrichment culture; —, unstable enrichment culture.

[c] *Eco*RI digests. Not conclusive results are either due to lack of digestion or due to variable results for different preparations.

[d] *Lac*1 and *Lac*2 are variants of *D. vulgaris* subsp. *oxamicus* Monticello. Because their genomes strongly cross-hybridize, they are referred to as a single standard, *Lac*1,2.

PB8-8 these are *Dec*7, *Pro*10, and *Ben*4, and for WW5-8 these are *Ben*3, *Pro*1, and *Ben*4 (Fig. 2 and Table 2). Since only one sample was collected for each site listed in Table 1, the terms "site" and "sample" can be used interchangeably; e.g., PB8 refers both to a site (Table 1) and to the sample collected from this site. DNA preparations PB8-1 to PB8-11 were obtained following growth of enrichment cultures on Pfennig's media with the indicated carbon sources (Table 2). Obviously, the labels for DNA preparations can also be used to indicate the enrichment cultures from which the DNA was prepared.

The status of liquid enrichment cultures can be deduced from Table 2, when multiple entries are provided for a given carbon source. For instance, sample WW3 grown on lactate contained *Lac*5 (WW3-1 and WW3-2) or *Lac*4 and *Lac*5 (WW3-3) and thus yields stable enrichment cultures. Colony purification also gave *Lac*5 (WW3-4, WW3-5, WW3-6, and WW3-7). These data consistently indicated that *Lac*5 is present at site WW3. Growth of sample WW3 on ethanol gave a mixed population (WW3-8: *Lac*4, *Lac*5, *Lac*10, *Ben*1, *Ben*4, *Dec*3, and *Pro*12), whereas single SRB were identified following liquid enrichment on benzoate (WW3-9: *Ben*1), decanoate (WW3-10: *Dec*3), propionate (WW3-11 and WW3-12: *Pro*12), and acetate (WW3-13: *Ben*1). The RSGP identifications of all enrichment cultures indicated *Lac*4, *Lac*5, *Lac*10, *Ben*1, *Ben*4, *Dec*3, and *Pro*12 as the SRB population at site WW3, and this has been entered in Table 1. It is noteworthy that if only lactate, the carbon source currently used most in SRB assays, had been used, a much more limited population (*Lac*4 and *Lac*5) would have been found.

A less straightforward picture was provided by sample WM10, grown on propionate. DNA preparation WM10-7 indicated the presence of *Ben*1 and *Pro*1, but WM10-8 indicated the presence of *Eth*2, *Dec*8, *Dec*7, and *Pro*1, and WM10-9 was identified as *Ben*4 and *Pro*1. Evidently, the composition of these enrichment cultures changed upon repeated transfer with *Pro*1 as a constant component. Moreover, colony purification using propionate as the carbon source (but possibly hydrogen as the electron donor as discussed below) gave neither of these standards. Both WM10-10 and WM10-11 were identified as *Lac*10. Although this was unexpected, it must be noted that *Lac*10 was

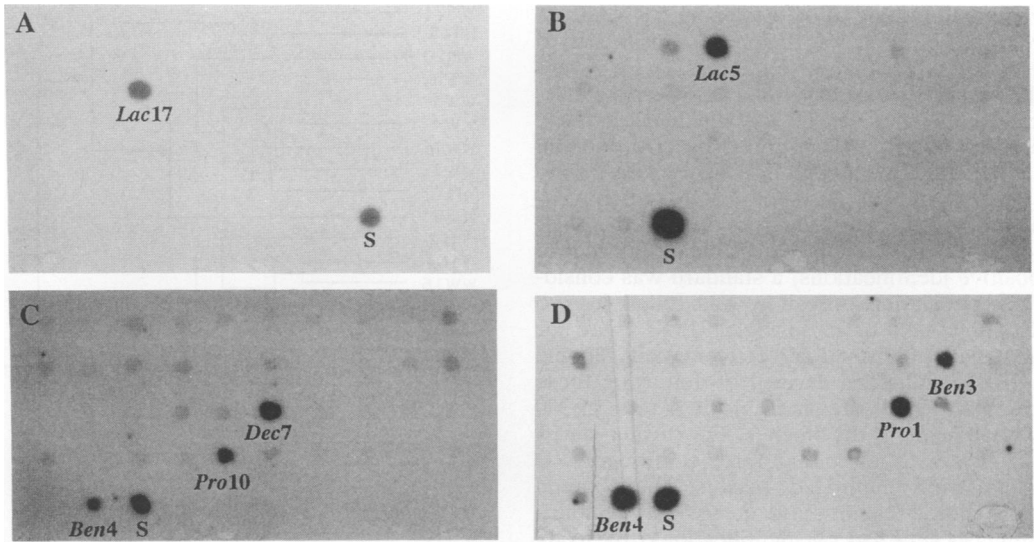

FIG. 2. Identification of SRB by RSGP. Denatured chromosomal DNAs of 35 different SRB standards (Table 3) were spotted on the master filters. The sample DNA preparation to be analyzed was spotted at the position indicated (S). The following sample DNA preparations were analyzed (Table 2): PB10-4, a colony-purified isolate of *Lac*17, using lactate as the carbon source (A); WM10-2, a colony-purified isolate of *Lac*5, using lactate as the carbon source (B); PB8-8, a liquid enrichment culture of sample PB8 using decanoate as the carbon source (DNA was isolated after one transfer [$T = 1$]) (C); and WW5-8, a liquid enrichment culture of sample WW5 using propionate as the carbon source (DNA was isolated after multiple transfers [$T > 1$]) (D).

present in enrichment cultures of the sample on lactate (WM10-1) and ethanol (WM10-4), indicating it to be a component of the SRB community at site WM10. Our interpretation of these results is that *Lac*10 is a minor component of the propionate enrichment culture, causing it to be undetected by RSGP. However, *Lac*10 readily forms colonies on propionate plates in a gas atmosphere containing hydrogen and is the only SRB detected so far under these conditions. Thus, the SRB standards predominating in a liquid enrichment culture did not necessarily predominate on solid media with the same carbon source.

Preparations containing sufficient DNA were also ana-

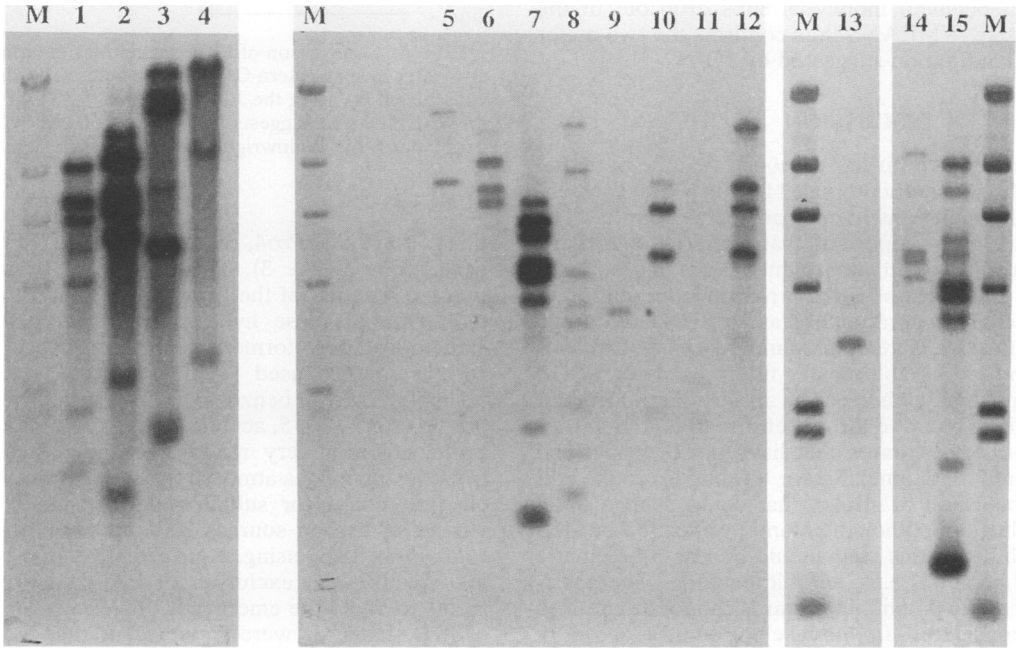

FIG. 3. Restriction enzyme fingerprinting of DNAs of some of the standards listed in Table 3. Following digestion of DNAs with *Eco*RI, gel electrophoresis, and Southern blotting, the blots were incubated with a 5′-end labeled 16S rRNA probe. Sample DNA preparations were as follows: M, size markers (from top to bottom, 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, and 0.56 kb); 1, *Lac*1; 2, *Lac*2; 3, *Lac*3; 4, *Lac*4; 5, *Pro*1; 6, *Pro*4; 7, *Lac*15 (Table 2, PB11-5); 8, *Lac*17; 9, *Lac*21; 10, *Pro*5; 11, *Pro*10; 12, *Dec*3; 13, *Lac*10; 14, *Lac*12; 15, *Lac*15 (Table 2, PB7-4).

lyzed by conventional probing using the genomic DNA from the 35 standards listed in Table 3 as the probe. This helped to clarify the presence of standards (e.g., *Lac*21) that were identified in the later stages of the project and were consequently absent from earlier versions of the master filter. RSGP identifications that were confirmed by conventional genome probing have been underlined in Table 2.

SRB composition at oil field sites. The RSGP and conventional genome probe data were used to deduce the SRB population at each site (see examples in Table 2). In order to reduce false-positive identifications, a standard was considered part of the population only if it was found in two independent RSGP assays or in a single RSGP assay for which the result was confirmed by conventional genome probing. The SRB populations derived by following these criteria are presented for all sites in Table 1. A total of 250 identifications were made at the 56 sites. For further statistical analysis 12 sites with fewer than three confirmed SRB standards per site were eliminated, because such limited population information does not allow meaningful comparison. The remaining 44 sites had 238 identifications (five to six different SRB standards per site). The population data were clustered by the program SYSTAT. The JOIN option of this program sorted the sites in a tree, such that sites with similar compositions are close together and are quickly connected into the tree. The tree derived for the 44 sites is shown in Fig. 4. It appears that sites at five of the six fields, Wainwright, Wildmere, House Mountain, Virginia Hills, and Harmattan, are scrambled into a single cluster, while the sites at the Pembina field form a separate cluster. The larger cluster is referred to as the Wainwright cluster, because samples analyzed from Wainwright make up the largest fraction. The tree shown in Fig. 4 is confirmed by the KMEANS option of the program, which clusters the sites into a specified number of groups to minimize overall dissimilarities. When two groups are specified, KMEANS clusters the sites identically as in Fig. 4. When more than two groups are specified, individual sites drop out of the Wainwright cluster. KMEANS thus confirms the two major clusters in the dendrogram suggested by JOIN.

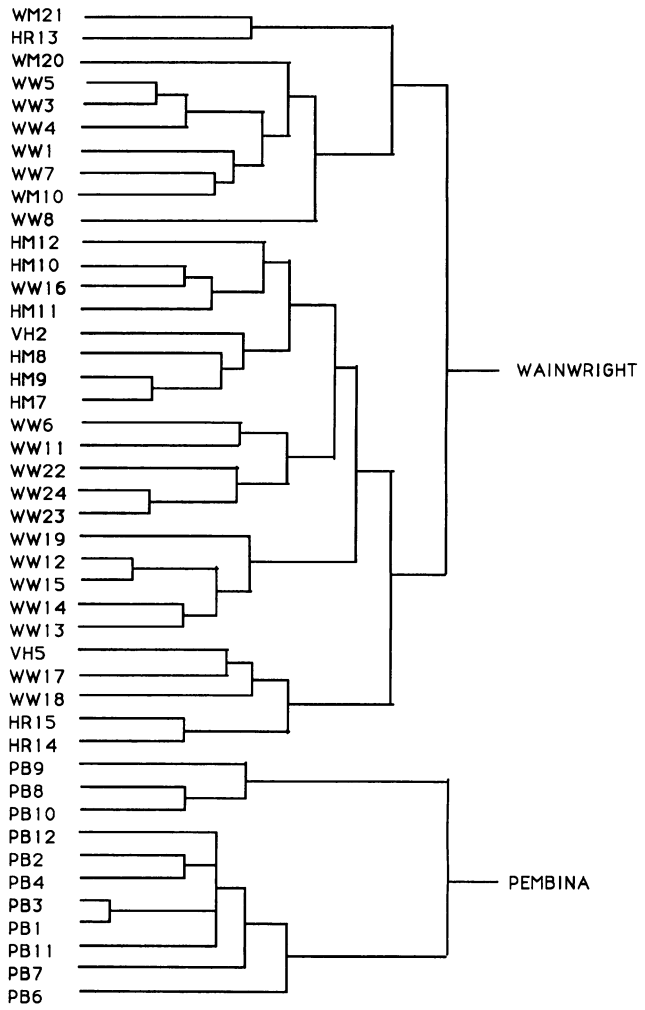

FIG. 4. Comparison of SRB populations determined with RSGP at 44 sites in six western Canadian oil fields. The similarity tree was constructed by using the JOIN option of SYSTAT, as explained in the text. The tree suggests two clusters of sites with different SRB populations, the Wainwright and Pembina clusters.

DISCUSSION

In this article we have further demonstrated the potential of RSGP, a method recently proposed for the general screening of microbial populations in environmental samples (17). For RSGP it is clearly important that bacterial standards, isolated from the targeted environment, are stored in a stabilized form that allows (i) their reproducible culturing and (ii) reproducible preparation of standard DNAs. Storage of stabilized cultures ensures that standard DNAs can continuously be spotted on the master filters used for RSGP identification and thus stabilizes the master filter formula. This was the main incentive for our efforts to colony purify the SRB standards. Standards that have not been purified beyond the liquid enrichment stage (Table 3) could still represent a consortium of strains that could change upon repeated subculturing. Although colony purification of SRB is notoriously difficult, this step should be less problematic for many other bacteria, e.g., aerobic bacteria. The possibility that many of the potential standards cannot be cultured should also be considered. In this case one could proceed by cloning large DNA fragments at random and spotting the amplified recombinant DNAs as standard representatives on a master filter.

DNA from all colony-purified standards, with the exception of *Lac*7 and *Pro*4, reacted with the [NiFe] hydrogenase gene probe (Table 3). This probe has been shown to be specific for SRB of the genus *Desulfovibrio* in earlier work (16). One of these hydrogenase-positive, colony-purified standards, *Lac*6, formed colonies on every one of the six carbon sources used (Table 2: VH2-2, lactate; HR14-7, ethanol; HM7-3, benzoate; HM7-6, decanoate; WW15-8, propionate; WW4-5, acetate). This result indicates that *Lac*6 forms colonies very readily, presumably using hydrogen from the mixed-gas atmosphere in the anaerobic hood as the electron donor for sulfate reduction while assimilating a variety of carbon sources. Colony purification of non-*Desulfovibrio* SRB, using, e.g., acetate as the electron donor, may require the exclusion of hydrogen from the plating media to avoid the emergence of *Desulfovibrio* colonies (2).

SRB standards were further characterized by hybridization with a total 16S rRNA probe. Hybridization intensity is likely to decrease with a decreasing degree of genetic relatedness of an SRB standard with *D. vulgaris* Hildenborough, the strain used for probe preparation. A phylogenetic tree,

based on 16S rRNA sequencing, has indicated that the SRB are genetically highly diverse (6, 7). Specific hybridization patterns were observed for 25 standards, whereas the results for 10 standards were inconclusive, either because of a lack of digestion or because of pattern variability. An example of the latter is standard *Lac*1,2 (Fig. 3, lanes 1 and 2), representing both *Lac*1 and *Lac*2, two variants of *D. vulgaris* subsp. *oxamicus* Monticello. The pattern observed for *Lac*15 showed limited variability (Fig. 3, lanes 7 and 15). Definition of 16S rRNA fingerprints is especially important for standards that have not yet been colony purified or obtained in a stable enrichment culture. The fingerprints allow evaluation of the possibility that new standards identified by RSGP are identical to these unstable standards, even after they have been dropped from the master filter formula. Another way of defining the standards that is currently pursued in our laboratory is to partly sequence the 16S rRNA genes following their amplification by the polymerase chain reaction (1). The sequences will suffer less from the restriction endonuclease polymorphism noted above, and their comparison with the existing data base for SRB (6, 7) may allow definitive identification of many of the standards listed in Table 3.

RSGP screening of enrichment cultures provides information on the types of bacterial standards present at a given location, not on their numbers. In this respect the technique differs from single-target gene probe methods, which are used to probe total-community DNA extracted from an environmental sample for the presence of a given bacterial species or the presence of a particular gene-encoded function (3, 9). Spotting known, increasing amounts of a positive control together with the nucleic acid samples being analyzed allows simple quantitation in single-target methods. Quantitative analysis by RSGP is more difficult and possible only if the culturing step is eliminated, but this is compensated for by the fact that many standards can be probed simultaneously. The possibility of quantitative RSGP without growth is currently under investigation in our laboratory.

Five of six oil fields from which samples were analyzed with RSGP (Wainwright, Wildmere, House Mountain, Virginia Hills, and Harmattan) have SRB populations that are similar and contrast to that found in the Pembina field (Fig. 4). The dendrogram presented in Fig. 4 is unusual in that it correlates populations at sites rather than individual bacteria. Dendrograms are frequently used to correlate bacteria via similarity in gene sequences (e.g., for 16S rRNA) or via shared genetic or phenotypic traits (e.g., the ability to use glucose or the capacity for sulfide production, etc.). However, one could consider the microbial consortium at an environmental site as an interacting community of which the individual members can be identified as traits. Dendrogram comparison of communities at sites by shared bacterial standards is then comparable to strain comparison by shared phenotypic traits. An important difference and limitation is that whereas the phenotypic trait analysis may be considered essentially error free, we cannot be equally certain that failure of a standard to appear in a liquid enrichment culture from a given site does indeed mean that it is absent from that site.

The distinctly different SRB population in the Pembina field can be highlighted by considering the frequency of occurrence of each standard in each of the two clusters (Table 4). Of 34 SRB standards, 10 appear only in the Pembina cluster while 18 are found only in the Wainwright cluster. There are only 6 organisms which occur in both clusters. It is clear from Fig. 1 that the five fields in the Wainwright cluster are not located in the same geographic area. However, a common feature is the high salt content of the aqueous phase of the oil-water mixture produced in these fields. For instance, at Wainwright and Wildmere, which produce heavy oil from the same geological formation, the produced water is on average a 6% (wt/vol) NaCl-rich brine, pH 7, free of H_2S and with a low free-sulfate concentration due to the presence of excess barium ions. Typical values for Na^+ and Cl^- were 15 and 27 g/liter for Wildmere and Wainwright, 18 and 29 g/liter for Virginia Hills and House Mountain, and 5 and 8 g/liter for Harmattan, while Pembina had only 1 and 1 g/liter. If the SRB population of the brackish Harmattan production waters is examined more closely, it is seen that *Lac*12 and *Lac*15 are detected in samples HR14 and HR15 (Tables 1 and 2). *Lac*15 was not isolated from the high-salt production waters but was found in two of three oil storage tanks (sites ED1, ED2, and ED3) that provided a freshwater environment to the resident SRB population. The SRB population in these tanks included one organism (*Lac*8) that has not been found in any other of the oil field samples listed in Table 1. It thus appears that salinity changes in the range of 2 to 50 g of NaCl per liter have a large effect on the resident SRB population in oil fields.

TABLE 4. Distribution frequency of 34 standards cultured from the saline Wainwright cluster of oil fields and the freshwater Pembina field[a]

Standard	No. of observations from:	
	Wainwright cluster	Pembina cluster
*Lac*4	13	
*Lac*5	8	
*Lac*6	24	
*Lac*10	9	
*Eth*3	6	
*Ben*1	26	
*Ben*3	2	
*Dec*1	9	
*Dec*3	10	
*Dec*8	3	
*Pro*1	5	
*Pro*4	9	
*Pro*7	1	
*Pro*11	1	
*Pro*12	5	
*Ace*1	9	
*Ace*3	5	
*Ace*4	4	
*Lac*1,2		2
*Lac*7		1
*Lac*17		2
*Ben*6		3
*Dec*4		5
*Dec*6		2
*Dec*7		1
*Pro*5		7
*Pro*10		4
*Ace*5		4
*Lac*3	4	2
*Lac*12	4	11
*Lac*15	1	7
*Lac*21	5	1
*Eth*2	1	2
*Ben*4	10	2

[a] The numbers presented are derived from the SRB population data in Table 1.

Cord-Ruwisch et al. (5) have described the resident SRB community in an oil treater near Hamburg, Germany, by enumeration of colonies forming on various media. They also indicated that salt had a strong effect on growth of the SRB in the population. Their data did not allow conclusions on possible population shifts. The treater had an NaCl concentration of 100 g/liter. The isolated SRB exhibited optimum growth at 10 to 50 g of NaCl per liter and were severely inhibited by salt concentrations in excess of 150 g/liter. The authors recommended that for effective control of SRB, injection waters of the highest available salinity should be used (5). Particularly severe microbial corrosion and souring problems in the freshwater Pembina field confirm the validity of this recommendation.

As shown here, elucidation of a community composition, even for bacteria that are as difficult to analyze as the SRB, can be readily achieved with RSGP. So far no other technique has reported the occurrence of so many different SRB in a comparatively large number of samples. Techniques that monitor specifically one type or class of SRB, e.g., by probing with a specific gene (16) or antibody (12), may fail to recognize some of the key players in this diverse community, which is generally held responsible for biological souring and corrosion problems in the oil and gas industry. For instance, the clustering of most of samples collected at Wainwright in 1989 (Table 1, WW1 to WW8) versus those collected in 1990 (Table 1, WW11 to WW19) indicates a shift in the SRB population of this field. The population data shown in Table 1 suggest the presence of acetate-utilizing SRB in the samples collected in 1990, which were not detected in the samples collected in 1989. We are presently attempting to correlate the information on the composition of SRB communities with physical and chemical data on corrosion rates at oil field sites.

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