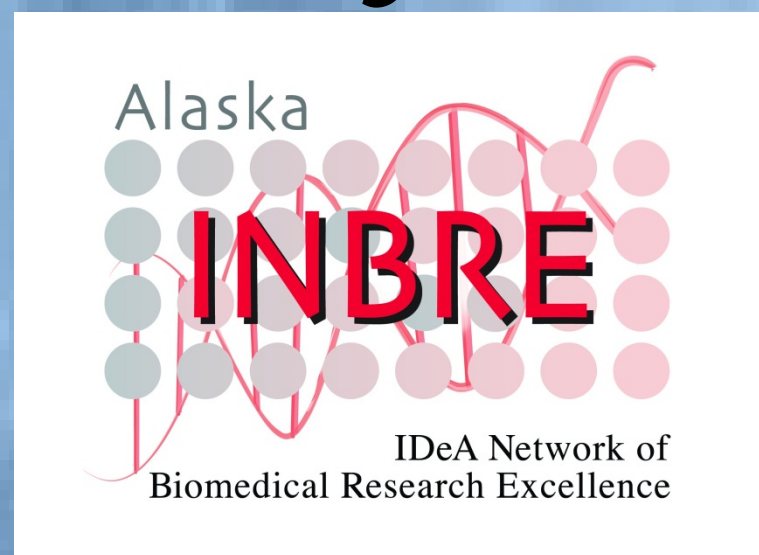


Analyzing Microbial Community Composition Within a Sulfolane Plume Using T-RFLP



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Introduction:

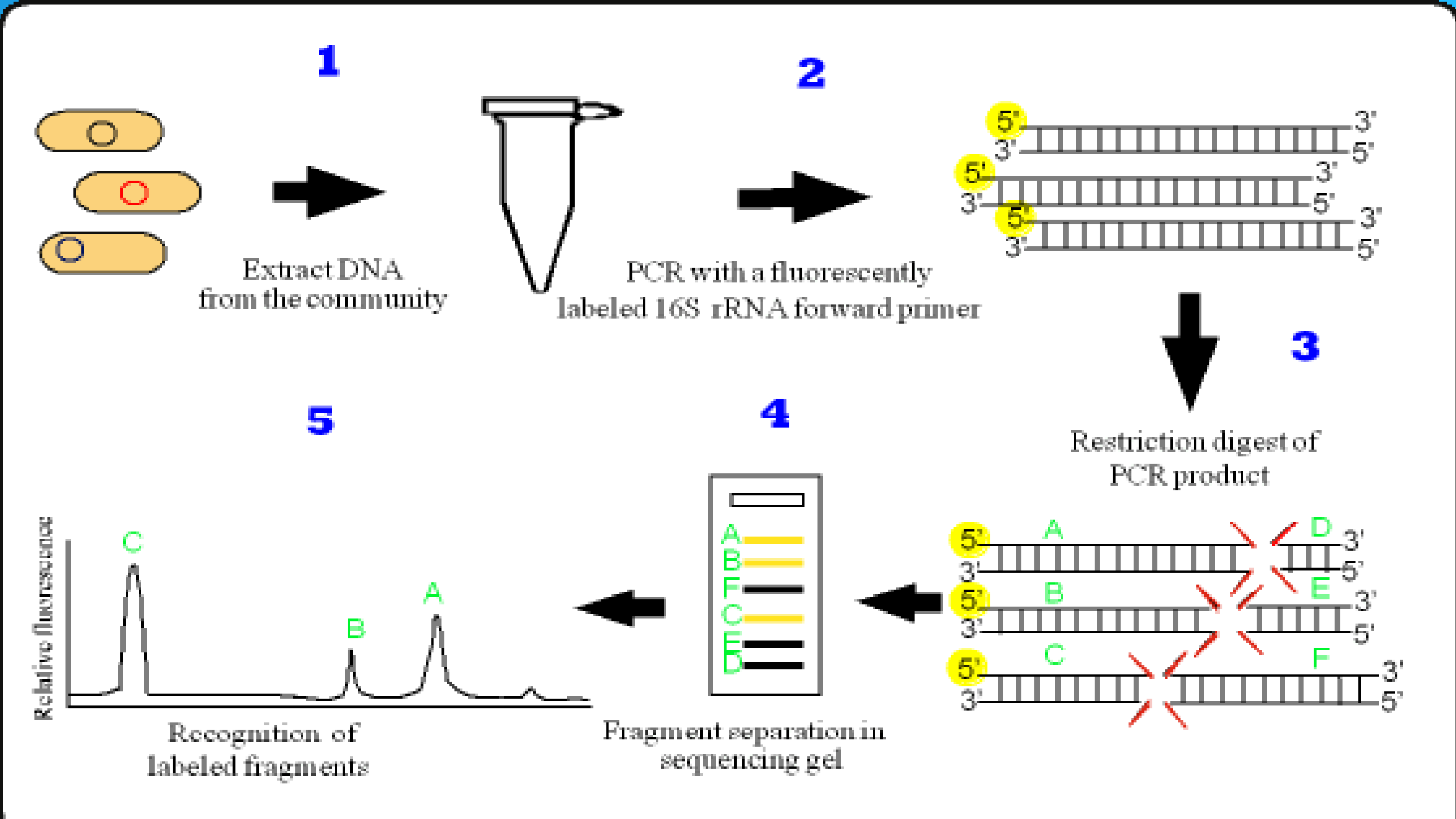
- Sulfolane is a highly water soluble man made chemical compound used in the process of oil refining and production.
- There has been a sulfolane spill at the Flint Hills Refinery in North Pole, AK, resulting in a sulfolane plume. Residential wells are located within the plume.
- Samples have been collected from monitoring wells (MW) on site in order to determine the effects of sulfolane on microbial communities.

Objectives:

- Compare groundwater microbial communities in samples of varying sulfolane concentrations and in air sparge (AS) treatment
- Identify microbial sulfolane degraders and evaluate their presence in groundwater samples

Methods:

- Samples were collected from groundwater monitoring and air sparging wells located within the sulfolane plume.
- The samples were added to liquid enrichment cultures with Sulfolane as their only carbon source. After several weeks, these cultures were plated onto general non-selective media.
- DNA was extracted from filtered samples
- PCR was conducted on isolates and DNA extracts using the fluorescently labeled forward primer 27F-FAM and reverse primer 1392R, which target the 16S rRNA gene.
- T-RFLP (Terminal Restriction Fragment Length Polymorphism) was run on purified PCR products.



<http://home.postech.ac.kr/~jaai/rflp-theory.htm>
Each TRF is assumed to be an indicator of a single species.

RESULTS:

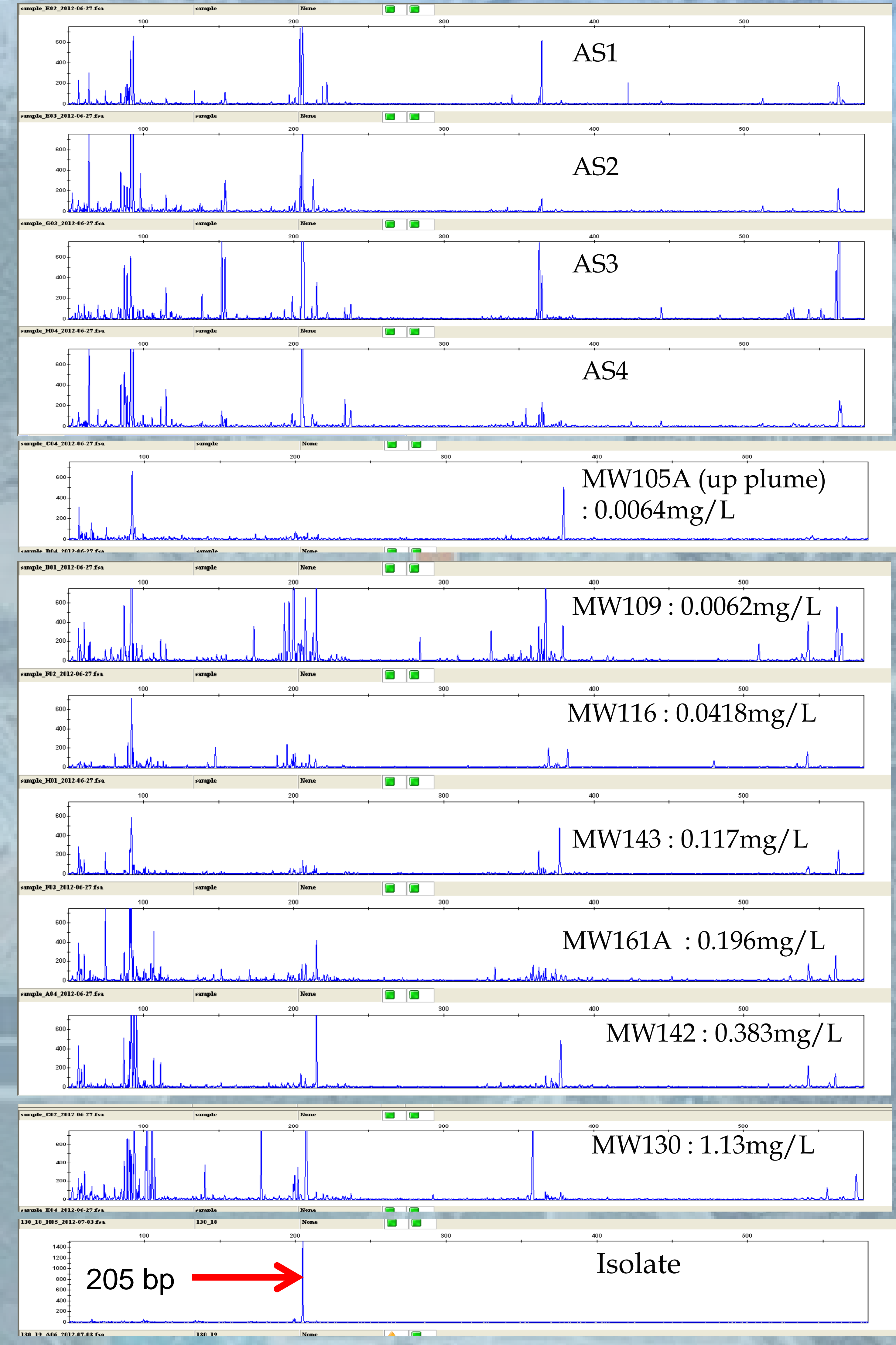


Figure 1: T-RFLP profiles of microbial community from AS and MW samples. AS samples show consistent similarities in community composition that are distinct from MW samples. The red arrow indicates the TRF of the isolate cultured in this study.

Sample	[sulfolane] (mg/L)	57	58	60	62	85	174	178	205	208
AS1	*	3.680	0.000	0.000	0.000	1.688	0.000	0.000	34.820	0.000
AS2	*	1.244	0.000	0.952	0.818	4.314	0.000	0.000	18.924	0.000
AS3	*	0.834	0.332	0.903	0.458	0.608	0.000	0.000	32.972	0.000
AS4	*	1.216	0.000	0.485	0.512	3.740	0.000	0.000	20.172	0.000
MW105	0.0064	13.182	3.023	5.122	0.000	0.000	2.141	0.000	0.000	2.729
MW109	0.0062	1.781	0.883	2.107	0.000	0.699	1.886	0.000	1.939	3.425
MW116	0.0418	0.000	1.571	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MW143	0.1170	7.446	5.545	3.931	0.000	0.000	0.000	0.000	5.233	2.239
MW161	0.1960	5.015	2.487	2.905	0.000	0.773	0.000	0.000	2.518	1.849
MW142	0.3830	4.595	2.975	2.734	0.000	0.000	0.000	0.000	1.654	1.080
MW130	1.1300	1.124	1.555	1.496	0.000	0.572	0.000	4.639	0.293	16.303

Table 1: Relative abundances of TRFs found in samples expressed as percentage of total peak heights. The size of the TRF (bp) is shown at the top. Blue indicates an air sparge sample. Green indicates an up plume sample. Yellow indicates moderate level of sulfolane concentration. Teal indicates a high level of sulfolane concentration. The orange box indicates the isolate. The red boxes highlight TRFs that differ between AS and MW samples.

Discussion:

- Certain TRFs were found exclusively in AS samples, while others were excluded from these and found only in MW samples. MW130 had extremely high sulfolane concentrations and contained several unique TRFs as well as some that increased in relative abundance (see table 1).
- The AS samples show consistent TRF profiles and are more similar to each other than they are to the other samples, indicating that air sparging treatment affects community composition.
- MW samples have many TRFs in common but abundances vary. In some cases large differences may be caused by sulfolane concentration (e.g., 174bp TRF above).
- A TRF of the same size as the isolate TRF (205bp) was found in almost all of the groundwater samples but was missing from the up-plume sample. This TRF makes up a larger proportion of the microbial community in AS samples.
- The aerobic environment of the AS system may lead to more efficient degradation of sulfolane by promoting the growth of aerobic sulfolane degraders such as the isolate from this study.

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