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Bioremediation of Petroleum-Contaminated Soil on Kwajalein Island: Microbiological Characterization and Biotreatability Studies

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6. FATTY ACID PROFILES

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6.1 INTRODUCTION

These experiments were designed to determine the profiles of ester-linked phospholipid fatty acids present in Kwajalein soil samples. The fatty acids are derived from microbial cell membranes, and the profiles obtained provide information regarding total biomass, community structure, and nutritional conditions of the microorganisms present. This information should help in the optimization of the conditions used for the demonstration project and should also be useful in monitoring the effects of bioremediation on the microbial population changes that occur in soil.

6.2 RESULTS AND DISCUSSION

The most reliable *in situ* technology for determining the viable or potentially viable microbial biomass in soils and sediments is the determination of the phospholipid ester-linked fatty acid content (PLFA). The presence of phospholipid in viable cells is based on the demonstrated metabolic lability of phospholipids in soils and sediments. The PLFA analysis does not require quantitative recovery of cells from the soil matrix (as do many direct counting procedures) or the quantitative growth of each isolate for viable counts. Table 6.1 indicates the presence of viable bacteria in the soils (and provides estimates of their numbers, based on 10^{12} cells/g and 100 μmol PLFA/g); similar trends were shown by both PLFA and plate count analyses.

Results of PLFA analysis also provide a quantitative estimate of the microbial community structure, as different subsets of the community have different PLFA patterns. In general, the profiles showed a high degree of similarity (Fig. 6.1). The presence of 10-methyl (mid-chain) saturated PLFA (10Me-16:0,18:0) indicated that aerobic actinomycetes were present, along with gram-positive organisms, from the short, terminally branched saturated PLFA. Samples tp4-w, tp4-65, tp5-62, and stp showed high levels of anaerobic desaturase PLFA (16:1w7c, 18:1w7c) characteristic of facultative heterotrophs. High levels of these PLFA correlated with low levels of the actinomycete signatures, which indicated a low to "absent" level of oxygen in these soils. All of the samples showed a low level of polyunsaturated PLFA and relatively little 18:1w9c, a precursor in aerobic desaturation.

Changes in the ratios of specific PLFA, primarily the ratios of trans/cis and cyclopropyl/monoenoic PLFA, indicate metabolic stress. Cyclo/mono ratios increase greatly with starvation and the stationary phase of growth. This ratio was highest in samples tp1-33 (which also showed an abundance of hydrocarbons), tp4-w, and tp4-32 (Fig. 6.2). Increased trans/cis ratios indicate starvation with exposure to a more toxic environment and were seen in tp1-33, tp4-w, tp5-62, and tp5-76 (tp4-w and tp5-62 also showed the highest levels of anaerobic desaturase PLFA).

Table 6.1. Viable or potentially viable cells

Sample	Concentration (pmol/g)	Cells/g	Plate counts
tp1-08	1012.3	5.9×10^7	6.0×10^6
-33	61.7	3.6×10^6	2.0×10^4
-56	30.7	1.8×10^6	3.5×10^4
tp2-06	65.2	3.8×10^6	1.6×10^5
-63	7.8	4.6×10^5	5.0×10^3
tp3-12	29.3	1.7×10^6	1.0×10^5
-72	377.0	2.2×10^7	1.2×10^3
tp4-32	822.5	4.8×10^7	3.0×10^5
-65	5.2	3.0×10^5	2.0×10^4
-w[a]	16.7	9.8×10^5	1.0×10^4
tp5-62	40.9	2.4×10^6	5.0×10^3
-76	10.4	6.1×10^5	4.0×10^1
stp[b]	5909.0	3.4×10^8	5.0×10^6

[a]Sewage treatment plant.

[b]Water.

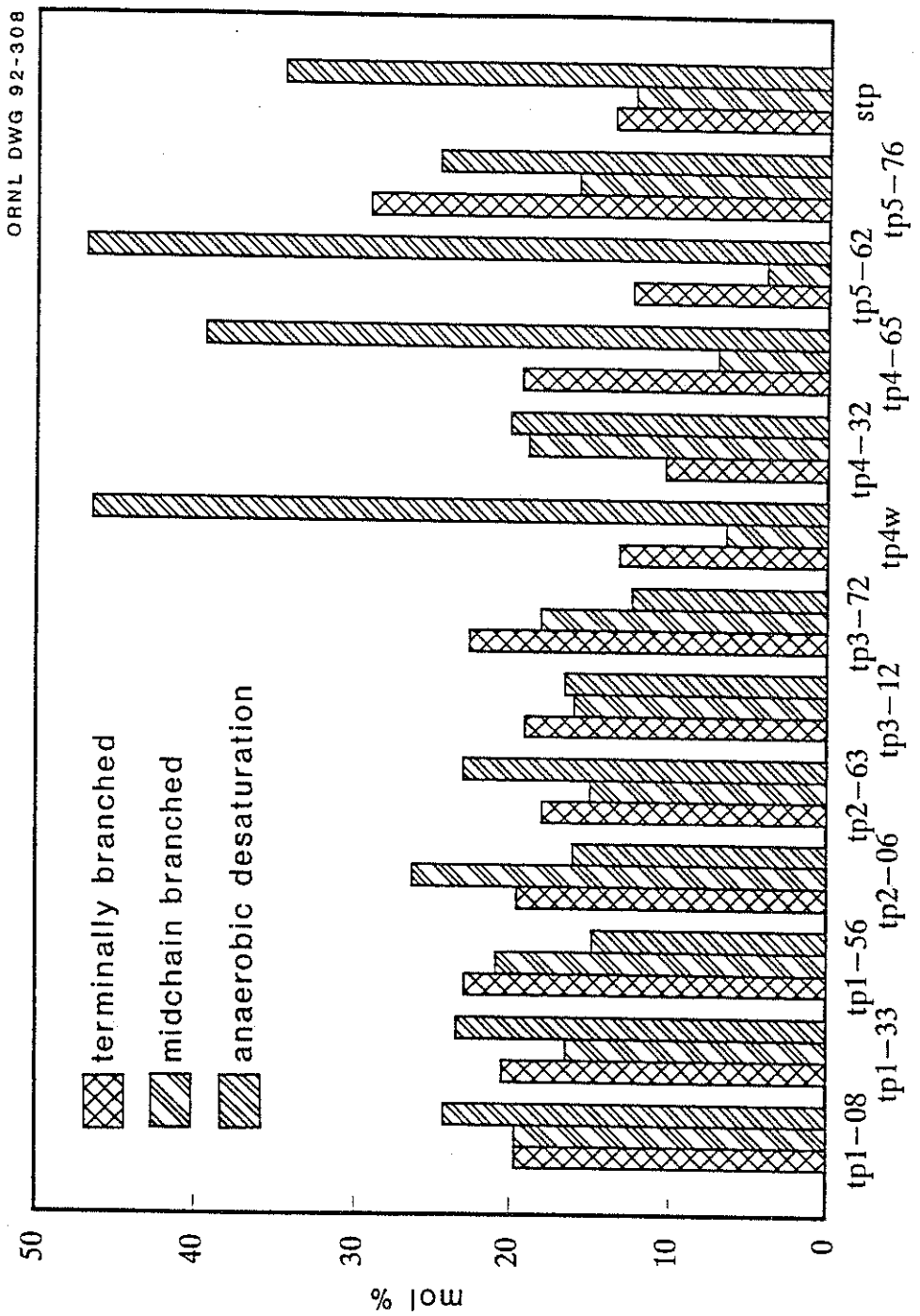

Fig. 6.1. Community structure from PLFA biosynthesis.

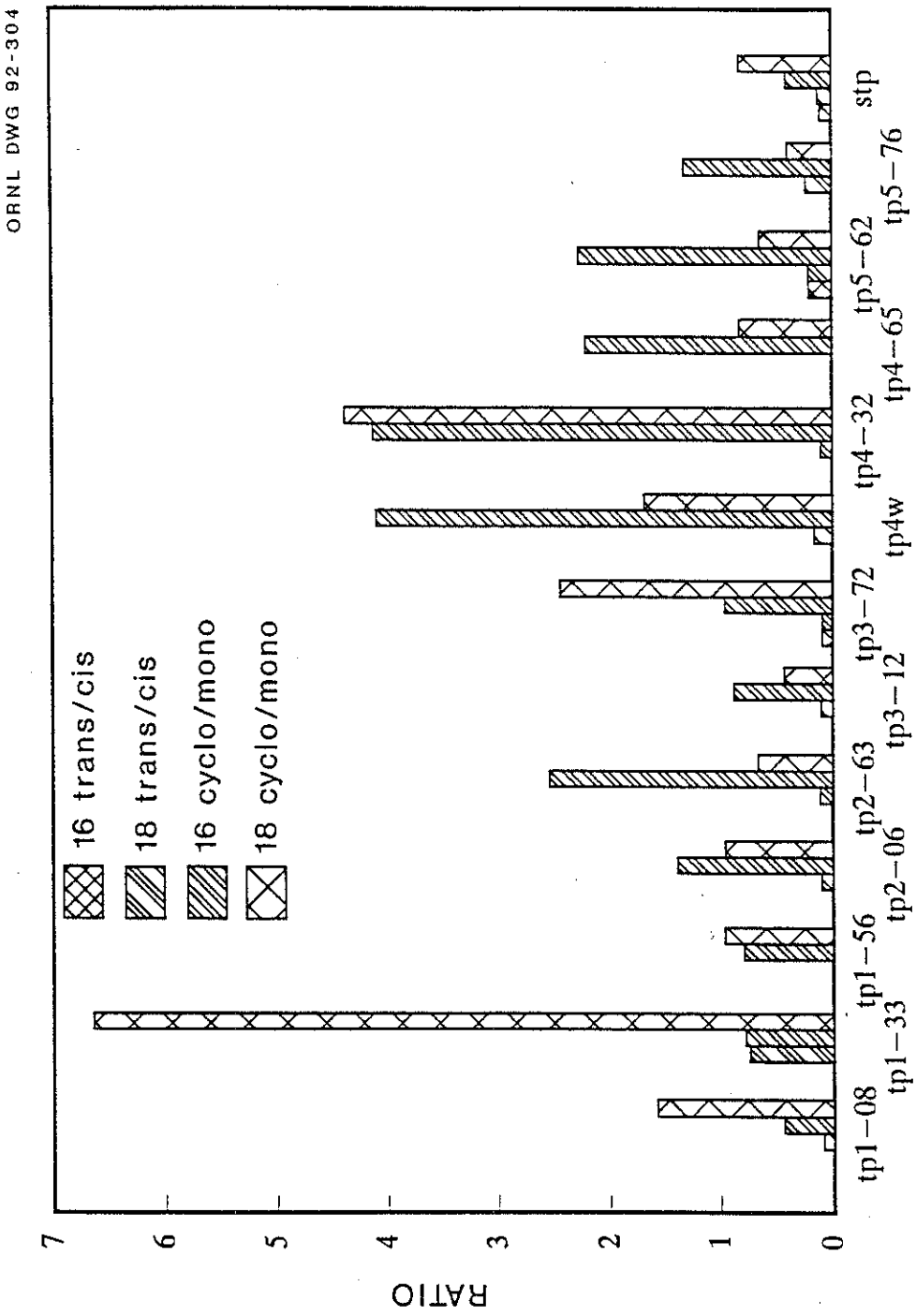

Fig. 6.2. PLFA as indicators of microbial physiology.

Formation of the endogeneous lipid storage product, poly beta-hydroxy alkanoate (PHA), defines conditions for unbalanced growth. This is readily demonstrated by the ratio of PHA/PLFA, which is an important measure of the community nutritional status as high levels are correlated strongly with the ability to degrade fortuitously metabolized contaminants such as TCE. The PHA detected in these samples was the butyrate polyester (PHB). The highest levels of PHB were found in samples tp4-32 and stp (Table 6.2).

Similarities between the total community in each sample can be quantitatively related with cluster analysis in which the most closely related patterns are grouped together (Fig. 6.3). The dendrogram shows two subgroups: the anaerobic desaturase PLFA and the branched-chain PLFA. The microbial community in sample tp4-32 was significantly distinct from either of these two groups, based on the presence of two unusual PLFAs. The dendrogram shows clearly that the communities in samples tp4-w, tp4-32, tp4-62, and tp4-65 are more similar to each other than to the other samples.

Table 6.2. Production of poly beta-hydroxy alkanoate

Sample	Concentration (nmol PHB/g)	PHB/PLFA ratio
tp1-08	20.36	20.11
tp3-12	5.34	182.25
-72	25.51	67.67
tp4-32	197.52	240.15
-65	4.76	915.38
-w[a]	37.60[b]	2251.50
stp[c]	249.82	42.27

[a]Water.

[b]nmol PHB/mL.

[c]Sewage treatment plant.

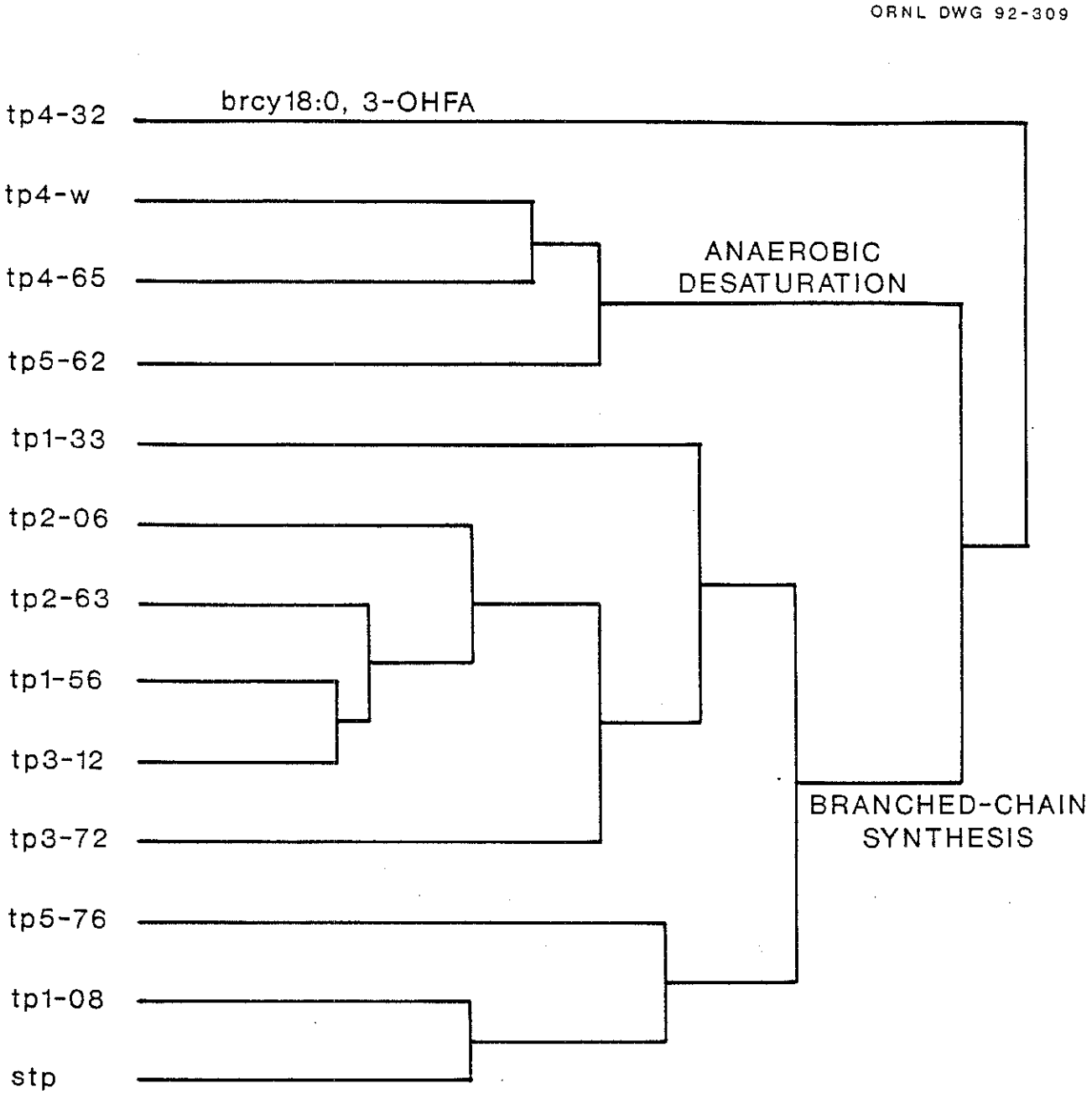

Fig. 6.3. Complete linkage: farthest neighbor cluster of sediment samples.