

Study of Piezophilic Bacteria in Biodegradation of Triacontane (C₃₀H₆₂)

Rifai Ridwan Muhamad

LabMET, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent, Coupure Links 653, 9000 Gent, Belgium

E-mail: ridwanrifai@windowslive.com

Piezophiles are bacteria that need high hydrostatic pressure for growth. They inhabit the deep sea, an oligotrophic environment where nutrient and carbon sources are limited. These conditions imposed over piezophiles a critical stress which allows them to make proficient use of some unique nutrient and carbon sources, as for instance aliphatic hydrocarbon. Some studies had been conducted to assess the response of piezophile on the contamination of aliphatic hydrocarbons.

Unfortunately, these studies did not reach hydrocarbons with a length of 30 atoms. Thus, here we examined the biochemical and microbial response of piezophilic bacteria in case of contamination by triacontane (C₃₀H₆₂) and identified bacteria able to degrade C₃₀H₆₂ and C₂₀H₄₂. Deep sea sediments from the West Iberian trench (1km, about 100bars or 10MPa) was enriched using three different pressure conditions (1, 100, and 200bars) and two oxygen conditions (aerobic and anaerobic) using C₃₀H₆₂ as the unique carbon source. The enrichment was conducted for nine consecutive incubation periods, each with duration of 10 days. Biochemical parameters (dissolved O₂, pH, and anions concentration) and microbial responses (cells number by flow cytometry and hydrophobicity response) were periodically measured during the enrichment. At the end of the enrichment step, the microbial community was analysed by using DGGE and Illumina gene sequencing.

Isolation procedures were conducted on microbial communities with a stable structure using culture dependent methods (i.e. plating w/o osmolites and high pressure reactors). Axenic isolates were identified by 16S rRNA sequencing and characterised using bioinformatics protocols. Bacterial growth was observed in each culture condition, carrying high respiration rates, showing acidification of the medium, and uptake of PO₄³⁻ and SO₄²⁻. DGGE analysis showed a microbial community shift following contamination with triacontane and Illumina gene sequencing later refined these results, showing a dominance of Acidobacteria, Gammaproteobacteria, and Alphaproteobacteria in the environmental sample. These dominant microbial groups were observed shifted towards Gammaproteobacteria and Epsilonproteobacteria; Gammaproteobacteria and Alphaproteobacteria; Deltaproteobacteria, Alphaproteobacteria, and Epsilonproteobacteria; and Deltaproteobacteria and Alphaproteobacteria in reactor 1AER, 1ANA, 100, and 200, respectively. As many as 37 single colonies were isolated and underwent Sanger sequencing, eventually resulting in 9 single strain sequences. RDP and EzTaxon gene database were used as references and generated 7 identified species names, i.e. *Pseudoalteromonas issachenkonii*, *Pseudoalteromonas undina*, *Photobacterium aplysiae*, *Vibrio kanaloae*, *Thalassospira povalitytica*, *Halomonas alkaliphila*, and *Halomonas titanicae*.

Meta-proteomics and biomass features analysis are underway to further understand the mechanisms and microbial cell properties featuring deep-sea solid hydrocarbons degraders.

Keywords: Piezophiles; microbial enrichment; microbial community shift; triacontane; hydrocarbons Biodegradation