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Isolation of 2-methyl-4-chlorophenoxyacetic acid degrading bacteria from groundwater sediments using a novel low substrate flux approach

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cultivated relative of Gmat59 was Ellin7146 with a SI of 88% over the 16S rRNA gene fragment analyzed. Gemmatimonadetes was successfully cultivated under both aeration conditions but was more frequently detected on plates incubated aerobically. Plates inoculated with tilled soil yielded more frequent detection of Gemmatimonadetes than plates inoculated with no-till soil.

239B Isolation of 2-methyl-4-chlorophenoxyacetic acid degrading bacteria from groundwater sediments using a novel low substrate flux approach

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The majority of Earth's biosphere consists of substrate-poor (oligotrophic) environments where the availability of carbon and energy sources is low. Cultivation-independent analysis has shown impressive bacterial diversity in these environments; however, cultivation of oligotrophic microorganisms remains challenging. Due to the fact that high substrate concentrations are commonly used for isolation purposes, bacterial populations existing in the same micro-niches that are adapted to metabolizing substrates at low concentrations may have been overlooked, and they could be more efficient at remediating low levels of organic pollutants. Herbicides are used worldwide for controlling weeds and their extensive use results in the presence of residues in surface- and groundwaters, so posing a threat to drinking water resources. 2-methyl-4-chlorophenoxyacetic acid (MCPA) is a phenoxyalkanoic acid herbicide that has been widely used for agricultural purposes. The weak retention of this compound in soil results in frequent occurrence in surface- and groundwaters. Here we present a novel cultivation approach aimed at isolating herbicide-degrading bacteria from an oligotrophic groundwater aquifer.

Saturated sediment samples were collected from a Danish groundwater aquifer. Enrichment cultures were established by inoculating sediment into a mineral medium with the herbicide MCPA as the only carbon source in low and high concentrations (100 µg L⁻¹ and 25 mg L⁻¹). A simple washing method was used to extract cells and fine particles from the aquifer samples without any prior enrichment. Direct extracts and enriched communities were cultivated by using a novel plate-based cultivation method, where low diffusive substrate fluxes are provided to cells growing on a membrane surface during extended incubation times for up to three months. Small colonies were then picked by a micro-manipulator and screened for their ability to mineralize ring-labelled ¹⁴C-MCPA. This approach provided numerous mixed communities composed of two to five bacterial strains and eventually led to the isolation of five different MCPA-degrading bacteria. These were identified by sequencing of 16S rRNA gene as well as dioxygenase genes (*tfdA*, *tfdA-like* and *cadA* genes). Furthermore, the ability of the bacterial strains to mineralize different MCPA concentrations was characterized and compared to the enrichment or extraction method by which they were obtained.

Using our novel method, we have successfully isolated five different MCPA-degrading bacteria affiliated with the genera *Achromobacter*, *Pseudomonas*, *Variovorax*, *Cupriavidus*, and *Sphingomonas*. The strains were different from each other in terms of MCPA mineralization at different concentrations, as well as the presence or absence of dioxygenase genes. The standard MCPA plates devoid of the membrane surface did not provide any MCPA-degrading strains, clearly indicating that the physical separation of growing bacteria and the agar medium by using a filter membrane reduces non-specific growth and allows for a more controlled and specific cultivation of degrader strains feeding on low flux of substrate.

059B Rarity and diversity of human-associated Archaea revisited - evidence of uncultured organisms related to Thermoplasmatales in human periodontal biofilms

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In contrast to many environmental ecosystems, Archaea constitute a clear minority within the human microbiome. Despite low abundance and diversity they have recently attracted novel attention due to increasing evidence of their (at least indirect) involvement in health and disease. With few exceptions