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Micro Fabricated Droplets Based Investigation for Soil bacterial Diversity

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Transitioning to a fully sustainable economy necessitates not only CO2-neutral energy production, but also closed loops in material processing,this challenge cannot be met solely through chemical conversion; biotechnology process steps must also be included, Its future development is dependent on the understanding and exploration of natural microbial resources, which are a priceless but largely undiscovered natural treasure, Conventional cultivation methods are limited in their ability to provide access to the vast unknown variety of natural microbial communities. Methods for high sample throughput, massive parallel cultivation, and testing of larger parameter spaces, in particular, are required.

Droplet-based microfluidics enables the parallelization of small cultivation volumes for large populations of microorganisms, On the one hand, it is possible to investigate the formation of microbial colonies by dilution of unknown communities down to the single-cell level using the principle of "stoachastic confinement," and on the other hand, it is possible to analyse the response of microbial populations to environmental pollutants, drugs, or even special substrates, The so-called "segmented flow technique" is particularly well suited for studying the concentration-dependent microbial response to special component concentrations, as it provides highly-resolved dose/response functions and maps of toxin combinatorial effects, These techniques have been used to examine soil samples from unusual environments such as mines, pyrometallurgical plants, and archaeological sites. As a result, very different soil bacterial communities have been discovered and characterised in terms of chemical response behaviour, new interesting species have been discovered, and new bacterial strains of known species with unknown special physiological properties have been separated, A variety of new strategies for culturing environmental microbes have been developed in recent years to increase throughput and recover taxonomically more diverse strains.

Overgrowth of fast-growing microbes is a significant impediment to effective culturing of rare or slow-growing microbes using traditional methods that employ solid or liquid growth media for culturing of cells in bulk, Cells can be stochastically isolated in discrete compartments prior to cultivation4,5,6 to overcome these challenges and recover a broader range of microbes, When compared to traditional methods, this eliminates resource competition and inhibitory effects among microbes and has been shown to generate isolates with increased taxonomic richness, including rare and clinically relevant taxa from the human gut, Regarding inoculum preparation (details in Methods), microbial cells were sequestered in over 500,000 nanoliter-sized w/o droplets, Cells were then cultured in a static environment for up to 8 days, and FNAP-positive droplets were recovered for further cultivation and identification, More specifically, after 2, 5, and 8 days of culturing, FNAP-positive droplets with proliferated microbes were recovered by on-chip FNAP-sort, with continued incubation of weakly fluorescent droplets sorted on days 2 and 5, The droplets' shape and size were examined using dark-field and fluorescence microscopy, as well as microbial growth in the droplets.

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