

Natural rubber degrading microbial communities: metagenomic assessment of the microbial diversity in the presence of rubber compounds

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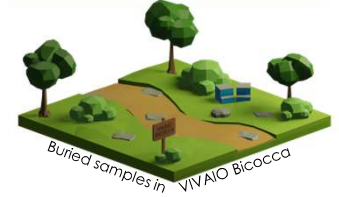
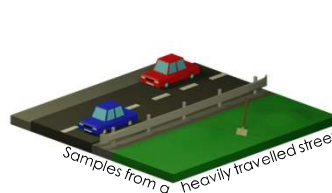
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Abstract

Natural rubber is a natural product, derived from *Hevea Brasiliensis* latex, and the main elastomer used in tires and rubber goods. Many studies in literature report the isolation of single strains capable of rubber degradation, but little is known about the microbial consortia able to lead such biodegradation. New approaches, such as metagenomics approach, have become a powerful tool to investigate biodiversity of soil microbial communities. Hence, our work aims to study the features of **microbial communities developed in the presence of rubber compounds** by understanding the structural and functional diversity present in rubber-contaminated soil with a metagenomic approach. Amplification of marker genes with high-throughput sequencing techniques will be performed for analyzing composition, richness, and biodiversity of soil bacteria in **rubber-polluted soil conditions**. A subsequent bioinformatic analysis through the QIIME software will provide a taxonomic classification and clustering of sequences in OTUs (operational taxonomic units). To **analyze soil bacterial diversity**, the alpha diversity (Shannon index) will be obtained by QIIME for each sample and, to compare the diversity among communities, beta diversity will be calculated using BrayCurtis (B-C) distance considering species' presence/absence and relative abundances. Through this approach, a clearer picture of microbial communities developed nearby rubber materials will be provided. Moreover, studies of the community's **rubber degrading enzymatic activities** will be further deepened in order to better understand the mechanisms of **rubber biodegradation** and exploiting them for **bioremediation processes** of contaminated sites.

Samples



Rubber-polluted soil was sampled beside the heaviest travelled street (>20.000 vehicles per day) in Lombardy and used as a source for rubber degrading microorganisms' isolation and analysis of its metagenome

Grids containing different rubber compounds were buried in 2021. Soil was sampled after two years (2023) and metagenome was extracted



SOIL SAMPLING

STUDY OF POLLUTED-SOIL MICROBIAL COMMUNITIES

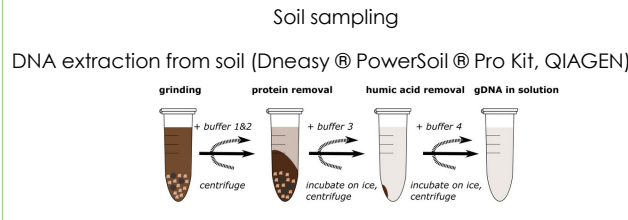
ISOLATION OF SINGLE RUBBER-DEGRADING MOOs

Workflow



BIODIVERSITY

Investigation of polluted-soil bacterial diversity



BIOREMEDIATION

Research of natural-rubber degrading enzymatic activities

Amplification of specific rDNA genes (PCR) and sequencing of amplicons

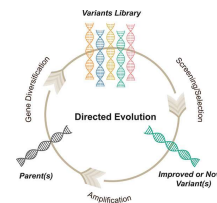


Sequencing and research of rubber-degrading enzymatic activities involved in rubber degradation from metagenomes and isolated microorganisms



Bioinformatic analysis of metagenomes through QIIME software packaging (Quantitative Insight Into Microbial Ecology)

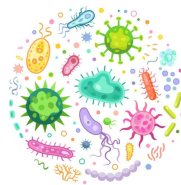
Taxonomy classification
Sequence clustering into OTUs



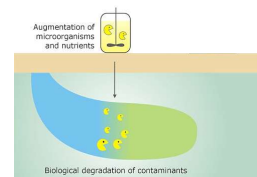
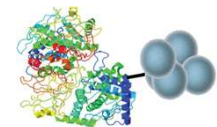
Optimization and evolution of enzymes from a single m.o. or a consortium

Identification of microbial diversity

- Alpha diversity (Shannon Index)
- Beta diversity (distance between the communities)



Enzyme immobilization for end-of-life rubber treatment



In situ application on roadsides (**Bioaugmentation**)

Acknowledgments