Biodegradation of Natural Rubber Through Enriched Microbiomes

Linking metagenomic insights, enzymatic activity, and material surface alterations

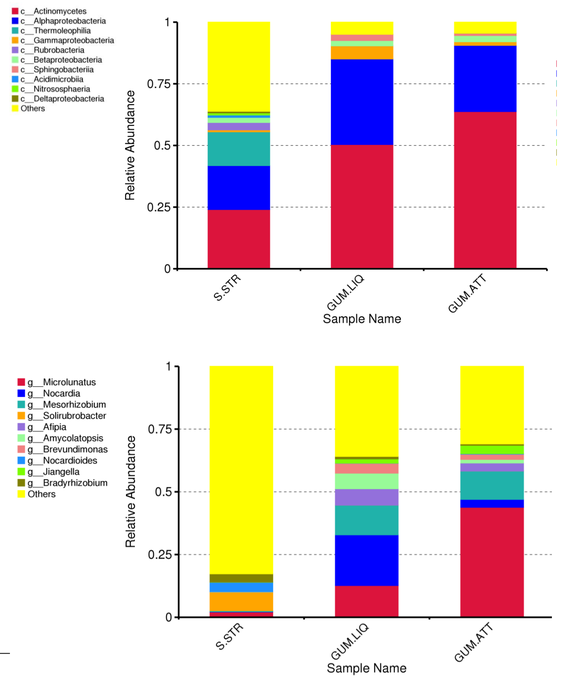
De Divitiis, M.[1,2], Bucchieri, D.[1,2], Dicesare, A.[1,2], Giannini L.[3], Guerra S.[3], Serra I.[1], Pérez-Garcia P.[4], Branduardi P[1]., Streit W. R.[4] [1],

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca Piazza della Scienza 2, 20126, Milano, Italy,
[2] Department of Material Science and Nanotechnology, University of Milano-Bicocca Via R. Cozzi 55, 20125, Milano, Italy,
[3] Pirelli Tyre S.p.A., Viale Piero e Alberto Pirelli, 25, 20126, Milano, Italy
[4] Department of Microbiology and Biotechnology, University of Hamburg, Hamburg, Germany

Taxonomy analyses of enriched rubber-degrading communities

Taxonomic profiling revealed a marked **shift in microbial community structure** following rubber-based enrichment.

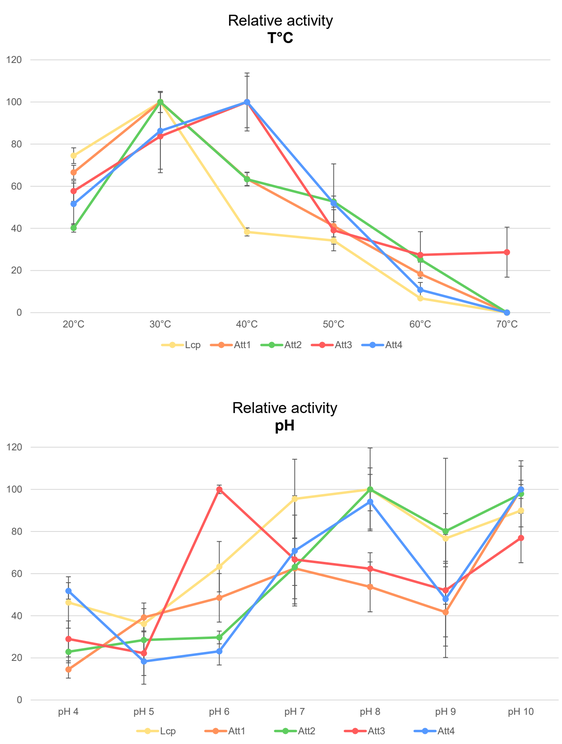
- The original soil sample (S.STR) showed a **more diverse** microbial composition
- Enriched samples (GUM.LIQ and GUM.ATT) exhibited **reduced taxonomic diversity** and a **shift in the community compositions:**
- Strong enrichment of **Actinobacteria**, especially the genus ***Nocardia***: ability to thrive on rubber-associated compounds, suggesting that rubber selects for specialized degraders
- Metagenomic reads were compared to NR database to annotate each metagenomic homolog (MEGAN)[1]



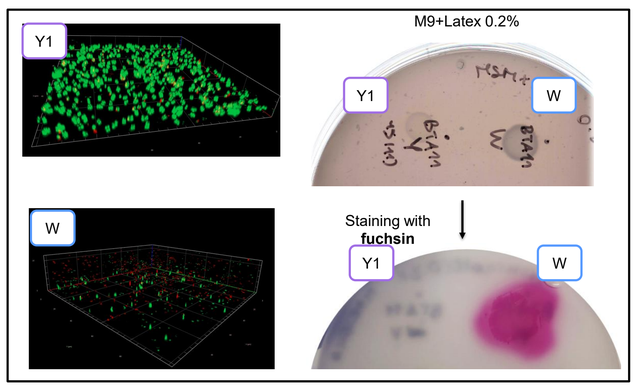
Enzyme mining and crude extracts characterization

- Screening for the KEGG ortholog **K23117** (Latex Clearing Protein, Lcp) identified 17 putative rubber oxygenase genes, with 12 in GUM.ATT. Full-length ORFs were extracted and filtered for completeness, leading to **4 high-confidence candidates (Att1–Att4), all from GUM.ATT**.
- These genes were cloned into pET24(+), expressed in *E. coli* T7 S. Huffle, and tested as crude extracts on natural rubber latex via a fuchsin-mediated colorimetric assay.
  All enzymes showed rubber-clearing activity.
- Optimal temperature differs across variants; Att3 and Att4 peak at 40°C and show higher thermal tolerance than Lcp, Att1, and Att2
  Enzyme activity varies widely with pH; Att3 stands out with a peak at pH 6 and broad stability from pH 6 to 10

These results highlight the **biotechnological potential of metagenome-derived Lcps** for rubber biodegradation under variable conditions.



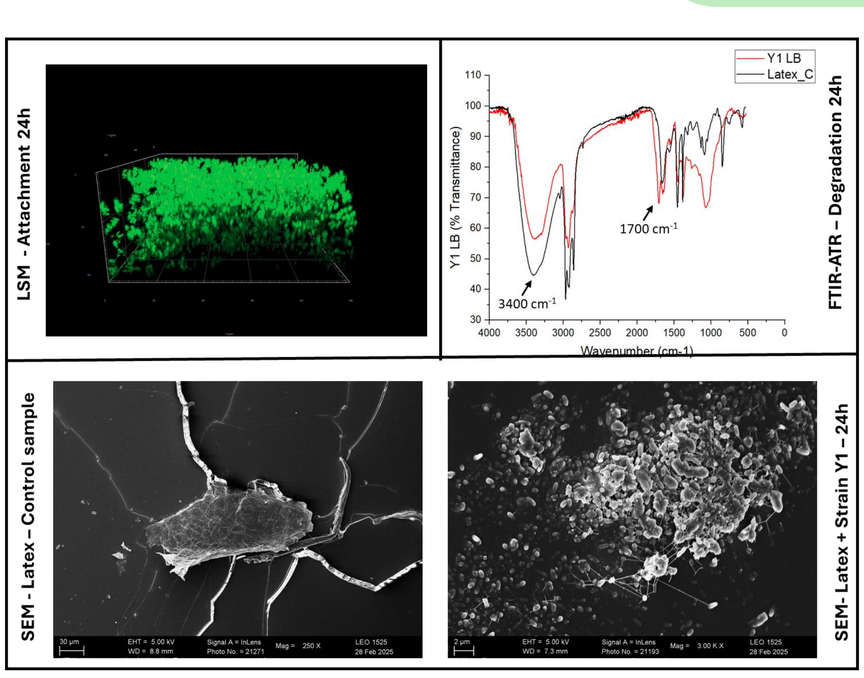
Microbial isolates: rubber degradation and biofilm formation



Two rubber-degrading strains were isolated using latex as the sole carbon source:

- Strain **W** (*Bacillus zanthoxyli*, Gram+, 99.06%) → halo formation on latex plates , extracellular oxidative activity
- Strain **Y1** (*Stenotrophomonas hibiscicola*, Gram–, 99.8%) → No halo formation, biofilm formation detected via LSM microscopy, surface-associated growth on rubber

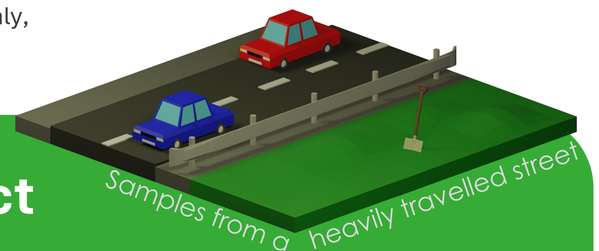
These strains represent the two main and complementary rubber-degradation strategies[2]



Characterization of strain Y1: early-stage (24h) **biofilm formation** and **degradative activity** on natural rubber latex film.

- Laser scanning microscopy (**LSM**): dense biofilm of viable cells.
- **FTIR-ATR. d**ecrease in the 3400 cm^{-1} band (O–H stretching) and emergence of the1700 cm^{-1} band (C=O stretching) suggest oxidative degradation of the polymer.
- Scanning electron microscopy (**SEM**): extensive microbial adherence to the latex surface

Strain Y1 is capable of rapid attachment and potential biodegradative activity.



Abstract

Natural rubber, derived from *Hevea Brasiliensis* latex, is the main elastomer in tires. While some rubber-degrading strains are known, little is understood about whole **microbial consortia**. This study investigates **communities enriched from rubber-contaminated soil** sampled beside the heaviest travelled street in Lombardy (SS32, >20.000 vechicles/day). **Enrichment** was performed by incubating the polluted soil with **films of both natural and commercial rubber**, then metagenomics was used to assess taxonomic diversity and to search for rubber-degrading enzymes. Enzyme mining via HMM identified **four active *lcp*-like genes**, cloned and expressed in *E. coli* T7 S.Huffle; extracts showed activity on natural rubber latex and were biochemically characterized. Shotgun metagenomics was used to perform taxonomy analyses of the communities, revealing an increase of well-known rubber-degrading species in the enriched communities. Two new strains were also isolated, able to form biofilms on rubber films. Moreover, the effect of microbial incubation on rubber films was evaluated using FTIR-ATR, and SEM, revealing significant transformations of the material. This integrative approach highlighted the microbial consortia associated with rubber materials and their enzymatic potential, paving the way for bioremediation applications in contaminated environments.

Conclusions

This study demonstrates that **enrichment of microbial communities on rubber** selects for specialized taxa, particularly Actinobacteria, known for their biodegradative capabilities. Functional metagenomics enabled the discovery of Lcp-like rubber oxygenases, which were cloned, expressed, and biochemically characterized, revealing diverse activity profiles and environmental tolerances. These results highlight the **strong biotechnological potential of metagenome-derived enzymes for rubber biodegradation** under variable and even extreme conditions. Additionally, two novel rubber-degrading strains were isolated, showcasing **distinct mechanisms of colonization and degradation**, including biofilm formation and oxidative activity. Altogether, the integration of omics, enzymology, and microbiology opens the way for the **development of sustainable strategies to valorize rubber waste** through targeted microbial and enzymatic interventions, with promising applications in environmental bioremediation.

References

1. Huson, Daniel H., et al. "Integrative analysis of environmental sequences using MEGAN4." Genome research 21.9 (2011): 1552-1560.
2. Jendrossek, Dieter, Gianpaolo Tomasi, and Reiner M. Kroppenstedt. "Bacterial degradation of natural rubber: a privilege of actinomycetes?." *FEMS Microbiology letters* 150.2 (1997): 179-188.

7th European Summer School on Industrial Biotechnology
Enzymes and polymers in the context of biocircular economy

Toulouse, 7.-10. July 2025

CONTACT ME!
m.dedivitiis1@campus.unimib.it
+39 345 220 4338

Marcella de Divitiis
Doctoral researcher at Università degli Studi di Milano-Bicocca & Pirelli ...

WEBSITE