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**Title:** Microbial Gutta-Percha Degradation Shares Common Steps with Rubber Degradation by *Nocardia nova* SH22a  
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**Abstract:** *Nocardia nova* SH22a, a bacterium capable of degrading gutta-percha (GP) and natural rubber (NR), was used to investigate the GP degradation mechanism and the relations between the GP and NR degradation pathways. For this strain, a protocol of electroporation was systematically optimized, and an efficiency of up to  $4.3 \times 10^7$  CFU per  $\mu\text{g}$  of plasmid DNA was achieved. By applying this optimized protocol to *N. nova* SH22a, a Tn5096-based transposon mutagenesis library of this bacterium was constructed. Among about 12,000 apramycin-resistant transformants, we identified 76 stable mutants defective in GP or NR utilization. Whereas 10 mutants were specifically defective in GP utilization, the growth of the other 66 mutants was affected on both GP and NR. This indicated that the two degradation pathways are quite similar and share many common steps. The larger number of GP-degrading defective mutants could be explained in one of two ways: either (i) the GP pathway is more complex and harbors more specific steps or (ii) the steps for both pathways are almost identical, but in the case of GP degradation there are fewer enzymes involved in each step. The analysis of transposition loci and genetic studies on interesting genes confirmed the crucial role of an alpha-methylacyl-coenzyme A racemase in the degradation of both GP and NR. We also demonstrated the probable involvement of enzymes participating in oxidoreduction reactions, beta-oxidation, and the synthesis of complex cell envelope lipids in the degradation of GP.

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