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# RoxB Is a Novel Type of Rubber Oxygenase That Combines Properties of Rubber Oxygenase RoxA and Latex Clearing Protein (Lcp)

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**Abstract:**

Only two types of rubber oxygenases, rubber oxygenase (RoxA) and latex clearing protein (Lcp), have been described so far. RoxA proteins (RoxAs) are c-type cytochromes of ≈ 70 kDa produced by Gram-negative rubber-degrading bacteria, and they cleave polyisoprene into 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD), a $C_{15}$ oligo-isoprenoid, as the major end product. Lcps are common among Gram-positive rubber degraders and do not share amino acid sequence similarities with RoxAs. Furthermore, Lcps have much smaller molecular masses (≈ 40 kDa), are b-type cytochromes, and cleave polyisoprene to a mixture of $C_{20}$, $C_{25}$, $C_{30}$ or higher oligo-isoprenoids as end products. In this article, we purified a new type of rubber oxygenase, $RoxB_{Xsp}$ (RoxB of *Xanthomonas* sp. strain 35Y). $RoxB_{Xsp}$ is distantly related to RoxAs with respect to molecular mass (70.3 kDa for mature protein) and cofactor content (2 c-type hemes). However, $RoxB_{Xsp}$ differs from all currently known RoxAs in having a distinctive product spectrum of $C_{20}$, $C_{25}$, $C_{30}$, and higher oligo-isoprenoids that has been observed only for Lcps so far. Purified $RoxB_{Xsp}$ revealed the highest specific activity of 4.5 U/mg (at 23 °C) of all currently known rubber oxygenases and exerts a synergistic effect on the efficiency of polyisoprene cleavage by $RoxA_{Xsp}$. RoxB homologs were identified in several other Gram-negative rubber-degrading species, pointing to a prominent function of RoxB for the biodegradation of rubber in Gram-negative bacteria. IMPORTANCE: The enzymatic cleavage of rubber (polyisoprene) is of high environmental importance given that enormous amounts of rubber waste materials are permanently released (e. g. by abrasion of tires). Research from the last decade has discovered rubber oxygenase A, RoxA, and latex clearing protein (Lcp) as being responsible for the primary enzymatic attack on the hydrophobic and water insoluble biopolymer poly(cis-1,4-isoprene) in Gram-negative and Gram-positive rubber-degrading bacteria, respectively. Here, we provide evidence that a third type of rubber oxygenase is present in Gram-negative rubber degrading species. Due to its characteristics, we suggest the designation RoxB for the new type of rubber oxygenase. Bioinformatic analysis of genome sequences indicate the presence of *roxB* homologs in other Gram-negative rubber degraders.

Keywords: rubber oxygenase, latex clearing protein, polyisoprene, biodegradation, heme dioxygenase, dioxygenases