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# Biodiversity of Bacterial lipase genes

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## Abstract

A number of bacterial species produce extracellular lipases. Among them, many lipase genes have been cloned and sequenced. A comparison of primary sequences revealed only very limited sequence homology among them. Based on the sequence homologies and molecular sizes ($M_r$), bacterial lipases were classified into four discrete groups. From soil samples taken around Taejon, five different lipase-producing bacteria were isolated; *Proteus vulgaris* K80, *Bacillus stearothermophilus* L1, *B. pumilus* B26, *Staphylococcus haemolyticus* L62, *S. aureus* B56. Nucleotide sequence analysis showed that *Staphylococcus* lipase genes (L62 and B56) composed of pre-pro-mature parts, *Bacillus* lipase genes (L1 and B26) pre-mature parts, and *Proteus* lipase gene (K80) mature part only. In addition, the molecular sizes of their mature parts were quite different from 19,000 to 45,000. Finally, they had very little homology (less than 20%) in their amino acid sequences. Judging from the above results, lipase K80 belonged to bacterial lipase Group I, lipase L1 and lipase B26 Group III, and lipase L62 and lipase B56 Group IV. This diversity in their primary structures was also reflected in their enzymatic properties; temperature effects, pH effects, substrate specificity, detergent effects, and so on.

## Introduction

Lipase catalyzes the hydrolysis of the ester bonds of triacylglycerols in oil/water interfaces. The enzyme was found widely in animals, plants, fungi, and bacteria. Among them, bacterial lipases were the most important in commerce and research not only due to their availability in large amounts but also their biodiversity and unique properties. A variety of lipases from Gram-negative and Gram-positive bacteria have been purified, biochemically characterized and the respective genes have been cloned and sequenced. Bacterial lipases were divided into four groups. Group I comprised most *Pseudomonas* lipases, Group II consisted of *P. fluorescens* and *Serratia marcescens* lipases. Group III and Group IV comprised *Bacillus* lipases and *Staphylococcus* lipases, respectively. A comparison of lipases from these four groups revealed that they were diverse in molecular size and that they had little sequence homology except for N-terminal oxyanion hole and the conserved sequences around catalytic triad (Ser-His-Asp). From soil samples taken around Taejon, five different bacterial lipases were screened. Here they were compared in the respect of molecular structures and biochemical properties.

### Lipase assay

Lipase activity was measured by titrating free fatty acids released by hydrolysis of olive oil using the pH-stat system (718 Stat Titrino, Metrohm).

## Results

Five different lipase-producing bacteria were isolated; *Proteus vulgaris* K80, *Bacillus stearothermophilus* L1, *B. pumilus* B26, *Staphylococcus haemolyticus* L62, *S. aureus* B56. The respective lipase genes were cloned and sequenced. Nucleotide sequence analysis showed that *Staphylococcus* lipase genes (L62 and B56) composed of pre-pro-mature parts, *Bacillus* lipase genes (L1 and B26) pre-mature parts, and *Proteus* lipase gene (K80) mature part only. Molecular sizes ($M_r$s ) of L62, B56, L1 mature enzymes were 43,000-45,000, whereas $M_r$s of K80 and B26 mature part were 31,000 and 19,000, respectively. In addition, they had very little homology (less than 20%) in their amino acid sequences. In spite of this diversity, they had some conserved regions of N-terminal oxyanion hole and around catalytic triad (Ser-His-Asp) except for B26 lipase. B26 lipase had conserved pentapeptide sequence of Gly(Ala)-X-Ser-Gly around active site Ser residue but had no conserved sequences around active site His and Asp. Sequence alignment with other known bacterial lipases demonstrated that lipase K80 belonged to bacterial lipase Group I, lipase L1 and lipase B26 Group III, and lipase L62 and lipase B56 Group IV. This diversity in their molecular structures was well correlated with their enzymatic properties. That is, these five lipases were found to have quite different temperature effects, pH effects, substrate specificity, detergent effects, and so on. Most striking biochemical features of each lipase were as follows. L1 lipase was thermostable and thermo-active enzyme, which was demonstrated by enzyme kinetics, fluorescence emission studies, and X-ray crystal structure. L62 lipase was detergent-stable and activated enzyme and cold-adapted enzyme. K80 lipase was alkaline enzyme. B26 lipase was $Ca^{++}$-independent enzyme and had partly esterase properties. These biochemical diversity observed in the above five lipases could be originated from the biodiversity of their molecular structure (primary structure).

## References

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