

*Full Length Research Paper*

# Classification of EC 3.1.1.3 bacterial true lipases using phylogenetic analysis

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**Lipases play an important role in lipid metabolism and are produced by a variety of species. All lipases are members of the  $\alpha/\beta$  hydrolase fold super-family. Also, lipases share a conserved active site signature, the Gly-Xaa-Ser-Xaa-Gly motif. To obtain an overview of this industrially and very important class of enzymes and their characteristics, we collected and classified bacterial lipases sequences available from protein databases. Here we proposed an updated and revised classification of family I bacterial "true" lipases based mainly on a comparison of their amino acid sequences and some fundamental physicochemical and biological properties. The result of this work has identified 11 subfamilies of "true" lipases. This work will therefore contribute to a faster identification and to an easier characterization and classification of novel bacterial lipolytic enzymes.**

**Key words:** Lipases, phylogenetic analysis, lipolytic enzymes.

## INTRODUCTION

Lipases are glycerol ester hydrolases that act on acyl-glycerols to liberate fatty acids and glycerol. Lipases can be divided into two main groups: (i) Carboxylesterases (EC 3.1.1.1) and (ii) "true" lipases (EC 3.1.1.3), which differ in several biochemical features. Lipase is an important class of enzymes with numerous applications in the rapidly growing biotechnology and biomedical fields. Lipases catalyze hydrolysis and synthesis of triglycerides and other water insoluble esters (Schmid and Verger, 1998), (Kazlauskas et al., 1998) (Figure1).

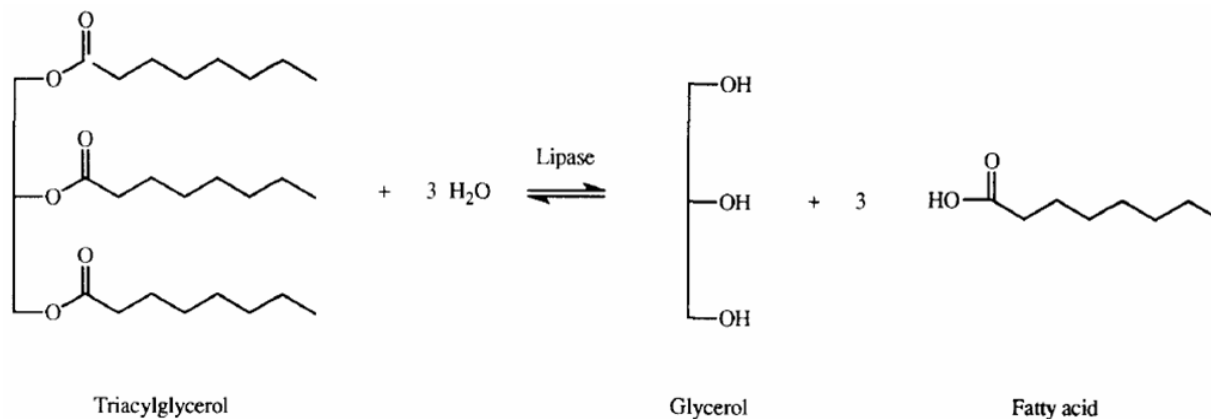
Lipases are ubiquitously produced by plants (Bhardwaj et al., 2001), (Belguith et al., 2009), animals (Carriere et al., 1994) and microorganisms (Olempska-Beer et al., 2006); microbial lipases is the preferred potent source due to several industrial potentials (Hasan et al., 2006). Therefore, there is an increasing interest in bacterial lipases because they represent the most versatile and widely used enzymes in biotechnological applications and organic chemistry (Reetz and Jaeger 1998). Most of the used lipases in industries are microbial enzymes, of both fungal

and bacterial origin; their applications can be found in many industries like pharmaceutical, dairy, detergent, cosmetic, oleochemical and others (Jaeger and Reetz, 1998).

Although lipases belong to many different protein families they have the same architecture, the  $\alpha/\beta$  hydrolase fold as defined by Ollis et al. (1992). Their activities rely mainly on a catalytic triad usually formed by Ser, His and Asp residues (Arpigny and Jaeger, 1999). In the  $\alpha/\beta$  hydrolases amino acid sequences, the three residues follow the order Ser-Asp-His. Also, lipases share a consensus sequence of Gly-Xaa-Ser-Xaa-Gly (Chapus et al., 1988; Osterlund et al., 1996; Saxena et al., 2003; Kanaya et al., 1998) where X may be any amino acid residue. Bacterial lipolytic enzymes are classified into eight families (families I – VIII) based on differences in their amino-acid sequences and biological properties (Arpigny and Jaeger, 1999). Family I of true lipases is the most represented one.

In the present paper, 53 sequences of bacterial lipases belonging to the family I known as true lipases, are compared and classified according to conserved sequence motifs and same biological and physico-chemical properties of these enzymes. This work presents an overview of bacterial lipases currently known and permits the classification of newly isolated lipolytic enzymes.

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**Figure 1.** Enzymatic reaction of a lipase catalyzing hydrolysis or synthesis of a triacylglycerol substrate.

## MATERIALS AND METHODS

Representative lipase sequences were collected from the ExPASy and the National Center for Biotechnology Information (NCBI) server. Precursor and putative sequences were removed to keep only mature and complete one. The BLASTP program was used for the sequence homology search in the database (version 2.2.15; Altschul et al., 1997). Sequence alignment was done using the ClustalW program (version 1.83; Thompson et al., 1994). The phylogenetic tree was drawn by Njplot software (Perrière and Gouy, 1996).

## RESULT AND DISCUSSION

Based on their amino acid sequences, we propose an updated classification of bacterial “true” lipases.

### *Pseudomonas* lipase subfamily

Based on their amino acid sequence homology, *Pseudomonas* lipases are classified into four subfamilies: I.1, I.2, I.3 and I.10 (Figure 2). This result conforms with that previously reported in literature (Jaeger and Eggert, 2002; Arpigny and Jaeger, 1999). One other study based on Biochemical properties and automatic sequence alignment classified *Pseudomonas* lipases into five subfamilies (Allan et al., 1995).

The subfamily I.1 includes lipases from *Pseudomonas aeruginosa*, *Pseudomonas fragi*, *Pseudomonas mendocina*, *Vibrio vulnificus*, *Proteus* sp. and *Yersinia enterocolitica*. The subfamily I.2 and I.10 hold lipases from the *Burkholderia* genus (previously part of *Pseudomonas* genus). Regarding subfamily I.3, bacterial lipases belongs to *Pseudomonas* sp., *Pseudomonas fluorescens*, *Acinetobacter* sp., *Psychrobacter* sp. and *Parvularcula bermudensis*. Subfamily I.1 and I.2 lipases share relatively high amino-acid sequence similarity and are secreted via the type II secretion system, in which they are first trans-

located across the inner membrane into the periplasmic space via the Sec pathway and then further translocated across the outer membrane via the secretion complex following chaperone-assisted periplasmic folding (Johnson et al., 2006).

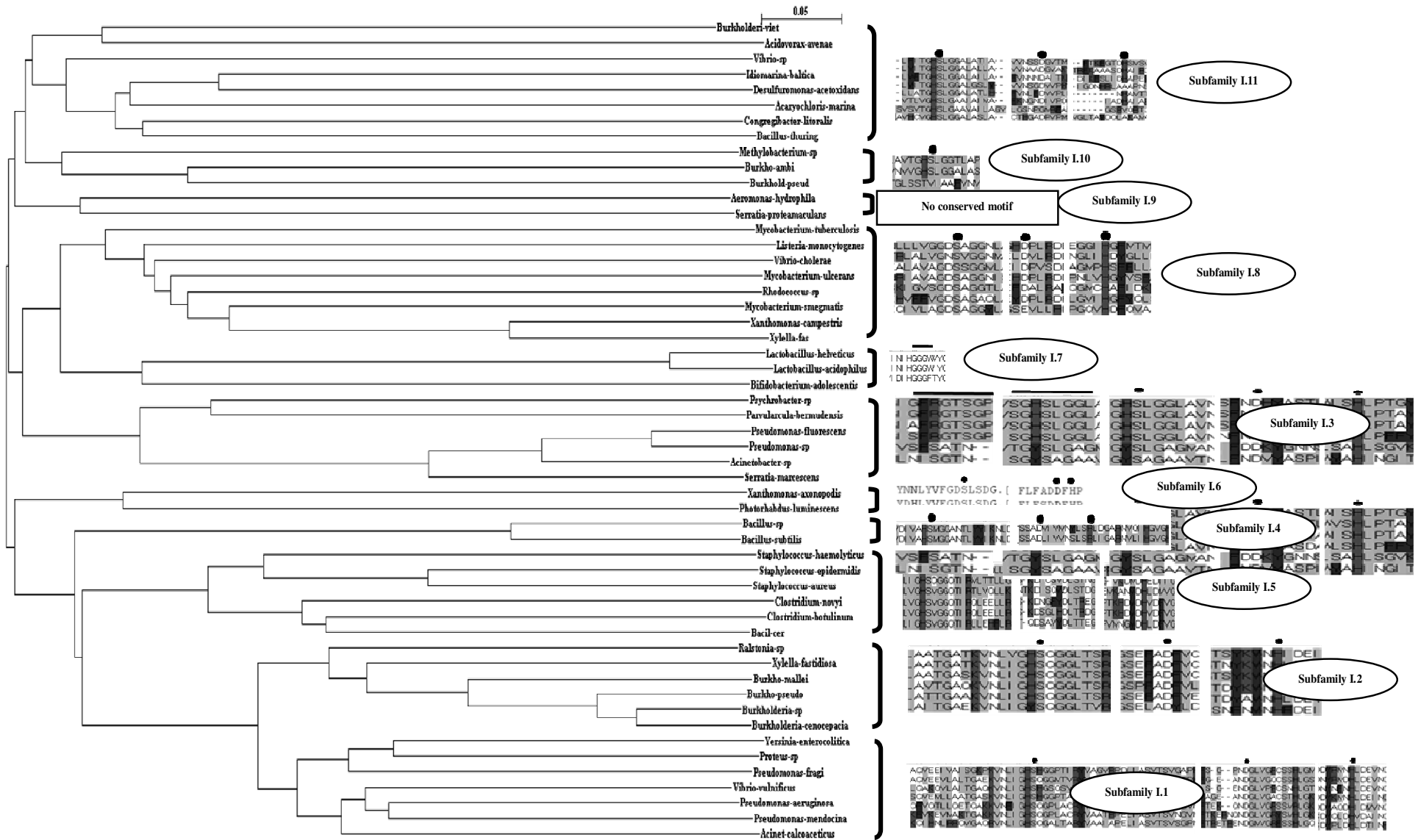
This bacterial gram negative true lipase subfamily is distinguished from other families not only by the amino acid sequence, but also by the secretion mechanism. Lipases of subfamily I.3 are secreted via the well-known type I secretion system. Like most proteins secreted via this system, subfamily I.3 lipases are composed of two domains with distinct yet related functions. Recent years have seen an increasing amount of research on this lipase family, in terms of isolation, secretion mechanism, as well as biochemical and biophysical studies (Angkawidjaja et al., 2006).

### *Bacillus* lipase subfamily

Due to their interesting biological properties, bacteria of the genus *Bacillus* are attractive candidates for a number of industrial applications (Wattiau et al., 2001). The *Bacillus* lipase show very little homology to the other lipases. Even the characteristic G-X-S-X-G sequence around the lipase active site serine residue has changed into an A-X-S-X-G sequence, and also the molecular mass (approximately 19 kDa) is much smaller than that of the lipase with known structures. *Bacillus* lipases belonged to either subfamily I.4 or subfamily I.5 (Figure 2). The first crystal structure of a member of homology family I.4 of bacterial lipases was in 2001 (Van Pouderooyen et al., 2001).

### *Staphylococcal* lipase subfamily

*Staphylococcus* lipases constitute a distinctive subfamily of microbial lipases that was named the lipase subfamily



**Figure 2.** Dendrogram representing an alignment of 53 lipases belonging to "true" lipase family. ●, amino acid residues belonging to the catalytic triad. Sequence alignment of each group are pointed out on the right tree side. Name of each subfamily are written in a white circle.

I.5. In addition, this family holds lipases from the *Clostridium* genus, and from *Bacillus cereus* species. Seong-Tae and collaborator confirmed that thermoalkalophilic lipases have significant sequence homology with *Staphylococcal* lipases, and he reported the first crystal structure of the lipase family I.5, a thermoalkalophilic lipase from *Bacillus stearotherophilus* (Seong-Tae et al., 2002)

### GDSL lipase subfamily

GDSL lipases represented by the subfamily I.6 are hydrolytic enzymes with multifunctional properties such as broad substrate specificity and regiospecificity. They have potential for use in the hydrolysis and synthesis of important ester compounds of pharmaceutical, food, biochemical and biological interests. This new subclass of lipolytic enzymes possesses a distinct GDSL sequence motif different from the GX SXG motif found in many lipases. Enzymes belonging to the GDSL family share five blocks of highly conserved homology which are important for their classification (Upton and Buckley, 1995). Studies show that GDSL hydrolases have a flexible active site that appears to change conformation with the presence and binding of the different substrates, much like the induced fit mechanism proposed by Koshland (Akoh et al., 2004).

### GGGX lipase family

The GGGX class is represented by the subfamily I.7. Fischer et al. (2006) confirmed that this class consists of 4 superfamilies with known protein structures, where the oxyanion hole-forming residue is located in a well conserved GGG pattern, which is followed by a conserved hydrophobic amino acid X. From sequence alignment and structure superposition, it was observed that the oxyanion hole is highly conserved inside the GX and the GGGX class (Pleiss et al., 2000). It was shown recently that this classification has important consequences for activity (Fischer and Pleiss, 2003).

### Marine bacteria lipase subfamily

Most members of the subfamily I.11 are lipases from marine bacteria sources. This group was described for the first time by Lee and colleagues in 2006, he confirmed that LipG which encoded a lipolytic enzyme, from a Korean tidal flat metagenomic library and other six putative bacterial lipases did not belong to any of the known lipase families. Therefore, he suggested that they comprise a new family of bacterial lipolytic enzymes (Lee et al., 2006).

### Other lipase subfamily

Sequence alignment of *Aeromonas hydrophyla*, and *Serratia proteomaculans* member of subfamily I.9 did not show significant conserved motif, which makes this group a target for further investigation and studies to explain the criteria of belonging to the lipases family.

Major members of the subfamily I.8 are lipases from *Mycobacterium* genus including *Mycobacterium ulcerans*, *Mycobacterium tuberculosis* and *Mycobacterium segmatis* species, which include the characteristic lipase consensus catalytic serine (Gly-X(1)-Ser-X(2)-Gly-) where X(1) is aspartate and X(2) is alanine (Figure 1).

### Conclusion

Lipases enzymes show a wide diversity of properties either on biochemical or molecular level. In this work we focused on true lipases family enzymes, and we tried to distinguish between subgroups in this large family and summarized the current knowledge available for each group.

### REFERENCES

- Akoh CC, Lee GC, Liaw YC, Huang TH, Shaw JF (2004). GDSL family of serine esterases/lipases. *Prog. Lipid Res.* 43(6): 534-552.
- Allan S, Kim B, Martin B, Tom BN, Erik G, Shamkant P (1995). Biochemical properties of cloned lipases from the *Pseudomonas* family *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabol.* 1259(1): 9-17.
- Arpigny JL, Jaeger KE (1999). Bacterial lipolytic enzymes: classification and properties *Biochem. J.* 343: 177-183.
- Belguith H, Fattouch S, Jridi T, Ben Hamida J (2009). Immunopurification and characterization of a rape (*Brassica napus* L.) seedling lipase, *Afr. J. Biochem. Res.* 3(11): 356-365.
- Bhardwaj K, Raju A, Rajasekharan R (2001). Identification, purification and characterization of a thermally stable lipase from rice bran. A new member of the phospholipase family. *Plant Physiol.* 127: 1728-1738.
- Carriere F, Thirstrup K, Hjorth S (1994). Cloning of the classical guinea pig pancreatic lipase and comparison with the lipase related protein 2. *FEBS Lett.* 388: 63-68.
- Chapus C, Rovey M, Sarda L, Verger R (1988). Minireview on pancreatic lipase and colipase. *Biochimie*, 70: 1223-1234.
- Fischer M, Pleiss F (2003). The Lipase Engineering Database: a navigation and analysis tool for protein families, *Nucl. Acid. Res.* 31: 319-321.
- Fischer M, Thai Q, Grieb M, Pleiss J (2006). DWARF a data warehouse system for analyzing protein families. *BMC Bioinform.* 7: p. 495.
- Hasan F, Shah AA, Hameed A (2006). Industrial applications of microbial lipases. *Enzyme Microb. Technol.* 39: 235-251.
- Jaeger KE, Reetz MT (1998). Microbial lipases form versatile tools for biotechnology. *Trends Biotechnol.* 16: 396-403.
- Jaeger KE, Eggert T (2002). Lipases for biotechnology *Curr. Opin. Biotechnol.* 13: 390-397.
- Kanaya S, Koyanagi T, Kanaya E (1998). An esterase from *Escherichia coli* with a sequence similarity to hormone-sensitive lipase. *Biochem. J.* 322: 75-80.
- Kazlauskas RJ, Bornscheuer UT (1998). *Biotransformations With Lipases.* Wiley-VCH, Weinheim, NY.
- Lee MH, Lee CH, Oh TK, Song JK, Yoon JH (2006). Isolation and characterization of a novel lipase from a metagenomic library of tidal

- flat sediments: evidence for a new family of bacterial lipases. *Appl. Environ. Microbiol.* 72: 7406-7409.
- Olempska-Beer ZS, Merker RI, Ditto MD (2006). Food processing enzymes from recombinant microorganisms-a review. *Reg. Toxicol. Pharmacol.* 45: 144-158.
- Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag JD (1992). The  $\alpha/\beta$  hydrolase fold. *Protein Eng.* 5: 197-211.
- Osterlund T, Danielsson B, Degerman E, Contreras JA, Edgern G, Davis RC, Schotz MC, Holm C (1996). Domain-structure analysis of recombinant rat hormone-sensitive lipase. *Biochem. J.* 319: 411-420.
- Perrière G, Gouy M (1996). An on-line retrieval system for biological sequence banks. 9084(96)84768-7. *Biochimie*, 78: 364-369.
- Pleiss J, Fischer M, Peiker M, Thiele C, Schmid RD (2000). Lipase Engineering Database understanding and exploiting sequence structure function relationships. *J. Mol. Catal. B-Enzyme*, 10: 491-508.
- Reetz MT, Jaeger KE (1998). Microbial lipases form versatile tools in biotechnology. *Trends Biotechnol.* 16: 396-403.
- Saxena RK, Sheoran A, Giri B, Davidson W (2003). Purification strategies for microbial lipases. *J. Microbiol. Methods*, 52: 1-18.
- Schmid RD, Verger R (1998). Lipases: interfacial enzymes with attractive applications. *Angew. Chem. Int. Ed. Engl.* 37: 1608-1633.
- Seong-Tae J, Hyung-Kwoun K, Seung-Jun K, Seung-Wook C, Jae-Gu P, Tae-Kwang O, Seong-Eon R (2002). Novel Zinc-binding Center and a Temperature Switch in the *Bacillus stearothermophilus* L1 Lipase. *J. Biol Chem.* 277(19): 17041-17047.
- Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.
- Upton C, Buckley JT (1995). A new family of lipolytic enzymes? *Trends Biochem. Sci.* 20(5):178-179.
- Van Pouderoyen G, Eggert T, Jaeger KE, Dijkstra BW (2001). The crystal structure of *Bacillus subtilis* lipase: a minimal  $\alpha/\beta$  hydrolase fold enzyme. May 25. *J. Mol. Biol.* 309(1): 215-226.
- Wattiau P, Renard ME, Ledent P, Debois V, Blackman G, Agathos SN (2001). A PCR test to identify *Bacillus subtilis* and closely related species and its application to the monitoring of wastewater biotreatment. *Appl. Microbiol. Biotechnol.* 56: 816-819.