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REVIEW PAPER

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Acinetobacter lipases: molecular biology, biochemical properties and biotechnological potential

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Abstract Lipases (EC 3.1.1.3) have received increased attention recently, evidenced by the increasing amount of information about lipases in the current literature. The renewed interest in this enzyme class is due primarily to investigations of their role in pathogenesis and their increasing use in biotechnological applications [38]. Also, many microbial lipases are available as commercial products, the majority of which are used in detergents, cosmetic production, food flavoring, and organic synthesis. Lipases are valued biocatalysts because they act under mild conditions, are highly stable in organic solvents, show broad substrate specificity, and usually show high regio- and/or stereo-selectivity in catalysis. A number of lipolytic strains of *Acinetobacter* have been isolated from a variety of sources and their lipases possess many biochemical properties similar to those that have been developed for biotechnological applications. This review discusses the biology of lipase expression in *Acinetobacter*, with emphasis on those aspects relevant to potential biotechnology applications.

Keywords *Acinetobacter* · LipA · Lipase · Biocatalysts

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Introduction

Lipases (EC 3.1.1.3) are best defined as carboxylesterases that catalyze both the hydrolysis and synthesis of long-chain acylglycerols [38]. True lipidic substrates are insoluble in water, and lipases commonly show activation upon contact with substrate micelles or emulsions, although they may also hydrolyze more soluble acylglycerols or monoester substrates. Microbial lipases have been studied for their role in virulence and their applications in biotechnology. Lipases are attractive biocatalysts because they act under extremely mild conditions, are stable in organic solvents, show low substrate specificity, and exhibit high regio- and/or enantioselectivity [35]. Their use in chiral synthesis of various pharmaceuticals and agrochemicals is growing, as is their penetration into detergent and food commodity markets. Lipases also serve as model catalysts to develop strategies for enhancing substrate enantioselectivity via directed evolution [36, 38, 39]. This versatility enhances the possibility of success in further development of existing technologies, as well as offering excellent promise for discoveries with novel applications.

Acinetobacter is a strictly aerobic, Gram-negative coccobacillus that is ubiquitous in geographical distribution. The genus is best known for its capacity for bioremediation of alkanes and aromatic hydrocarbons, as well as production of high molecular weight heteropolysaccharides that act as powerful emulsifiers, many with high commercial potential [41, 62, 69]. *Acinetobacter* strains have also been identified as causative agents of nosocomial infections [63]. They are easily isolated and many of them have been found to secrete esterolytic enzymes. However, description of these catalysts and their development as industrial biocatalysts has lagged behind that of *Pseudomonas*/*Burkholderia* lipases, despite having been isolated from the same environment. This may be because the latter were the first to be studied, but also may be a result of the con-

fusing history of *Acinetobacter* taxonomy [29]. Nevertheless, interest in *Acinetobacter* lipases has increased recently, with the growth of the enzyme industry and the concomitant widening search for novel enzymes and applications. Here, we review the literature on *Acinetobacter* lipases, with emphasis on their biotechnological importance.

Occurrence of lipolytic strains

Lipolytic strains of *Acinetobacter* have been isolated from a variety of substrates, including human skin, dairy and other food products, in addition to diverse soil and water habitats, both pristine and highly polluted (Table 1). Clinical strains are often lipolytic, causing severe nosocomial infections in neonates and immunocompromised patients [24, 33, 63]. The lipase activity, exopolysaccharide production, and cell surface hydrophobicity of hospital isolates may play an important role in their virulence [4, 32]. Lipase production by psychrophilic bacterial strains (predominantly pseudomonads) isolated from preprocessed dairy products have been found to be the cause of souring or spoilage of these foods during storage [18, 19].

Lipolytic strains are also commonly isolated, along with pseudomonads, from waste water effluents and sewage, where they may be continually exposed to petroleum pollutants and xenobiotic compounds [1]. In such environs, lipase activity has been correlated with hydrocarbon utilization [72]. This situation is paradoxical because hydrocarbons are nonlipase substrates and their degradation does not require lipase activity [47]. Moreover, some alkanes, such as hexadecane, have been shown to repress lipase expression (measured as β-galactosidase activity in a *lipA::lacZ* fusion strain) [47]. Thus, the explanation for lipase production by *Acinetobacter* strains in the presence of alkanes remains elusive and requires further study.

Table 1 Occurrence and isolation of lipolytic *Acinetobacter* strains

Strain	Source	Description	Reference
Various	Freshwater (polluted)	Mesophilic/ psychrophilic	[9]
O_{16}/O_4	Freshwater	Psychrophilic/ mesophilic	[11, 12]
69 V	Unknown		[23]
SY1, IB2, BO2	Activated sludge		[15]
BD413	Soil	Produces high MW bioemulsifier	[41, 44]
RAG-1	Seawater	Produces high MW bioemulsifier	[50, 69]
OPA 55	Olive oil		[56]
AAAC323-1	Soil	BD413 derivative	[10]
CMC-1	Soil	Produces high MW bioemulsifier	[31]
LP009	Raw milk	Psychrotrophic	[21]
KM109	Soil		[60]
16265	Clinical		[32]
Strain No. 6	Soil	Psychrotrophic	[78]
Various (50)	Clinical		[33]
SY-01	Water sludge		[30]
Various	Oil-contaminated soils		[55]

Biochemical properties

Following the classification proposed by Arpigny and Jaeger [3], lipases produced by *Acinetobacter* spp. are true lipases belonging to subfamily I.1. They share many biochemical properties with other *Pseudomonas*/*Burkholderia* group lipases that have been described, including *Pseudomonas aeruginosa* [27], *Pseudomonas fragi* [2], *Proteus vulgaris* [42], and *Burkholderia cepacia* [40]. Molecular mass reports vary (Table 2), but DNA sequence data [45, 77] predict mature proteins approximating 32 kDa, characteristic of the family [3]. Most are highly hydrophobic in nature, even in comparison with other bacterial lipases, as evidenced by the number of purification and recovery methods that exploit this characteristic [10, 31, 45, 52, 75, 84].

Many *Acinetobacter* lipases show stability and maximum activity at alkaline pH, a useful characteristic in detergent applications (Table 2). High pH optima reported in studies of lipase A_1 from *A. radioresistens* stimulated exploration of technologies designed to enhance production and yield that would be appropriate for large-scale production required in such applications [16, 51, 52]. Activity at acidic pH (ca. pH 5.0) is minimal [31, 45, 75], presumably due to titration of the active site histidine. Incubation at lower pH results in inactivation, which Lang et al. [49] attributes to loss of Ca^{2+} via titration of its coordinating residues. In addition, these lipases demonstrate broad substrate specificity typical of microbial lipases that have been shown to be useful in attacking mixed fat stains. Specificity toward short [30], medium [66, 75], and long [31, 45] fatty acid monoesters (*p*-nitrophenyl), and even benzoyl esters [60], has been reported.

A near universal property of *Acinetobacter* lipases is the positive effect of Ca^{2+} on enzyme stabilization and activity (Table 2), most probably a function of the Ca^{2+}-binding pocket [45, 77], leading to correct active-site configuration [49]. Calcium binding has been demonstrated in crystallized *P. aeruginosa* lipase, the subfamily prototype enzyme [61]. Furthermore, analyses of structural data from the closely related family I.2 clearly show a commonality of the Ca^{2+}-binding [43, 49, 64, 74] and strongly suggest its conserved nature throughout the family. The effect of Ca^{2+} may also be attributed to interaction with the assay medium, precipitation of free fatty acids, or otherwise increased enzyme access to the substrate [13, 27, 34, 53].

The effect of metals and inhibitors has been studied with respect to suitability of lipases for industrial application. Generally, incubation in the presence of metals has little effect on lipase activity and most likely

Table 2 Biochemical properties of purified *Acinetobacter* lipases. *LCFA* Long chain fatty acids, *DMSO* dimethylsulfoxide, *DMF* dimethylformamide

Strain	MW (kDa)	Cloned	pH optimum/ stability	Temperature optimum (°C)	Inhibitors	Description	Reference
A. calcoaceticus AAC323-1	NR[a,b]	No				Stabilized by Ca^{2+}	[10]
A. calcoaceticus BD413	32	Yes	7.8–8.8/NR			Specificity toward LCFA esters; activity and yield increased by Ca^{2+}	[45]
A. radioresistens CMC-1	45	No	10.5/8–11	40	Zn^{2+}, PMSF	Alkaline lipase; specificity toward LCFA esters; activity increased by DMSO and DMF	[17, 31]
Acinetobacter nov. sp. KM109.	62	No	8/6–8	45		Hydrolyzes benzoate esters	[60]
A. calcoaceticus LP009	23	Yes	7/4–8	50	EDTA, acetonitrile	Activity restored by Ca^{2+}	[21, 66]
Acinetobacter sp. O_{16}	≥200	No	7.5/NR	35		Purified as high MW aggregate; activity increased by Ca^{2+}	[11, 12]
Acinetobacter sp. RAG-1	33	Yes	9/6–9	55	EDTA, pyridine	Specificity toward C_6, C_8 fatty acid esters of *p*-nitrophenyl, stabilized by Ca^{2+}	[75]
Acinetobacter sp. SY-01	43.8[c]	Yes	10/9–11	50		Enantioselective; specificity toward C_2–C_6 fatty acid esters of *p*-nitrophenyl, activity increased by Ca^{2+}	[30]

[a]Not reported
[b]Derivative of BD413, MW assumed to be 32 kDa
[c]Contained 40 amino acid signal sequence

depends on specific reaction conditions, rather than general properties of the enzyme. Incubation in the presence of EDTA demonstrated a variable dependence on Ca^{2+} for activity, i.e., 70% loss in activity occurred 8 h post incubation with the chelator [10]. Addition of Ca^{2+} after exposure to EDTA resulted in reactivation of Lip009, presumably by stimulating refolding of the enzyme [66]. However, other investigators have found no "rescue effect" of Ca^{2+} incubation post exposure to EDTA [75]. These seemingly contradictory findings suggest that Ca^{2+} may be required for prolipase folding, in addition to activity of the mature lipase. Addition of phenylmethylsulfonylfluoride (PMSF) to lipase preparations also yielded mixed results [21, 31, 75]. Dharmsthiti et al. [21] hypothesized that the deeply recessed nature of the active site serine imparts resistance to serine hydrolase inhibitor. Incubation of purified enzymes in the presence of reducing agents [2-mercaptoethanol, dithiothreitol (DTT)] resulted in activity that was not dependent upon intact disulfide linkage [21, 31, 75]. Since the presence of disulfide bridges has been confirmed in related crystallized proteins, these results suggest a more important role in the interaction with their cognate lipase-specific foldase, or Lif, during folding and export. It is quite plausible that, during purification, lipases aggregate with various cellular materials, i.e., emulsifying agents, lipopolysaccharides (LPS), or other materials that confer protection from potential inhibitors [12, 75, 76].

Stability in the presence of organic solvents is a requisite property of enzymes used in organic synthesis in non-aqueous systems. *Acinetobacter* lipases appear to be ideally suited for such syntheses since many lipolytic strains have been isolated from petroleum-polluted environments [9, 31, 69, 83]. Incubation of purified A_1 lipase in either 40% (v/v) dimethylsulfoxide (DMSO) or 20% (v/v) dimethylformamide (DMF) greatly increased activity (140% in DMSO) [31], leading to the proposal that solvents act to decrease enzyme aggregation, modify the substrate-water interface, or convey positive conformational changes. However, storage of Lip009 in solvents for 24 h (4°C) resulted in significant deactivation [21]. In contrast, lyophilized RAG-1 preparations retained significant enzyme activity in non-polar solvents, presumably because of the increased rigidity of the molecule [75].

Expression, regulation and secretion

Lipases belonging to subfamilies I.1 and I.2 are encoded in an operon together with their cognate Lif chaperone. The *lif* chaperone is usually encoded downstream from the structural gene, with the exception of *A. calcoaceticus* BD413, where the reverse occurs [46]. Co-expression of lipase genes (*lip*) with their cognate foldases is required for secretion of the mature lipase.

Mechanisms regulating lipase expression in *Acinetobacter* have been described only in strain BD413 and its derivatives. Expression of the lipase structural gene (*lipA*), measured in a BD413-derived *lacZ* transcriptional reporter, was found to be repressed by long-chain

fatty acids (LCFA) released in triolein degradation [47]. The mechanism proposed to explain this phenomenon was mediation of *lipA* expression via an unidentified fatty acid-acyl-responsive DNA binding protein activated when free fatty acids were bound [47]. Oleic acid used as a sole carbon source was found to have a significantly negative effect upon *lipA* transcription over the entire concentration range (0–10 mM) tested [54]. Lipase repression by fatty acids has also been reported in *P. aeruginosa* [28]. Growth on hexadecane may repress lipase expression [47]. *A. calcoaceticus* BD413 showed strong induction of lipase activity only upon cessation of growth on hexadecane, suggesting that hexadecane, or one of its degradation intermediates, represses *lipA* expression [47].

A transcriptional *lipA* :: *lacZ* fusion was also used by Nudel et al. [65] to study the effect of iron limitation on lipase production in BD413. They observed that iron-starved cells grown in minimal medium showed a slight decrease in *lipA-lacZ* transcription, whereas addition of tryptone (1%) resulted in a 4-fold increase in exocellular lipase activity. These findings indicate regulation of extracellular lipase activity by iron occurs post transcription of the structural gene [65].

Regulation of BD413 extracellular lipase activity also occurs through proteolytic degradation [47]. Rapid deactivation of lipase activity in cell-free extracts of N-broth cultures occurred 90 min after the culture reached stationary phase [44, 47]. Degradation of the lipase protein was confirmed by immunodetection and attributed to the presence of an endogenous serine protease [47]. A rapid decrease in lipase activity, with cessation of exponential growth, was reported in strain RAG-1 grown on minimal medium supplemented with various triglycerides [75]. However, enzyme stability was significantly increased in both strains when the production medium contained hexadecane as the sole carbon source [47, 75]. Protection from proteolysis may be conferred when the lipase is associated with hexadecane micelles [47].

Secretion of microbial lipases belonging to subfamilies I.1/I.2 takes place via the two-step, type II secretion pathway [81]. The prolipase is transported across the cytoplasmic membrane by Sec proteins in a signal-dependent manner, followed by secretion through the outer membrane via Xcp protein machinery [81]. Absence of Xcp-like protein complexes in *Escherichia coli* prevents cloning and expression in that system [30, 45, 77]. Efficient lipase expression has been demonstrated in other hosts, including *Aeromonas sobria* [21] and *Bacillus subtilis* 168 [30].

Barbaro et al. [6] observed that a rapid reduction in growth temperature (25–5°C), i.e., in "cold shocked" *Acinetobacter* sp. HH1-1 cells, resulted in decreased lipase export to the supernatant phase. This finding was explained by alteration of membrane export proteins, accompanied by decreased membrane fluidity caused by the cold shock [5, 6]. However, production of extracellular lipase can be increased by addition of polysaccharides, such as gum arabic, to the growth medium. Lipase yields from *A. cacoaceticus* BD413 cultures were improved up to5-fold in minimal medium containing lactic acid and gum arabic, compared to those grown in lactic acid alone [54]. Polysaccharides may aid in lipase secretion by freeing cell-bound molecules accumulated at the cell surface [54, 86].

Expression of a mature lipase requires Lif accessory protein activity [37] and Dsb general folding catalytic activity [59]. Lifs contain hydrophobic *N*-terminal sequences that anchor the foldase to the cytoplasmic membrane, whereas the hydrophilic *C*-terminus protrudes into the periplasm. Interaction between lipases and their cognate Lifs appears to be specific. El Khattabi et al. [22] observed that *E. coli* DH5α expressing *P. aeruginosa* or *Burkholderia glumae lipA* with non-cognate *lif*s did not produce active lipase. Further, co-expression of *P. aeruginosa lif* and *A. calcoaceticus lipA* failed to produce lipase activity in the same host [22]. Although Lif helper proteins are believed to be required by lipolytic *Acinetobacter* strains, such a requirement has been demonstrated only for strains RAG-1 and BD413 [46, 77].

Sequence comparisons of lipase subfamilies I.1 and I.2

The phylogenetic tree predicted from multiple sequence alignment of prolipases belonging to subfamilies I.1 and I.2 supports division of this family based on conserved sequence motifs and biochemical properties [3] (Fig. 1). However, Fig. 1 shows subfamily I.1 can be further divided into three clades. For consistency with past nomenclature, the first is named the "*P. aeruginosa*" clade, a designation used by Gilbert [26] and Jaeger et al. [37]. Its members have greater than 60% amino acid (aa) identity and include lipases from *Pseudomonas* sp. 109, *Pseudomonas aeruginosa* PAO1, *Pseudomonas pseudoalcaligenes* M—1 and *Vibrio cholerae*. The second group, originally named the *Pseudomonas fragi* family by Svendsen et al. [79], also includes lipases from *Proteus vulgaris* K80 and *Pseudomonas fluorescens* C9, having greater than 44% aa identity. The last subgroup, the "*Acinetobacter*" clade, contains lipases from *Acinetobacter venetianus* RAG-1, *Acinetobacter calcoaceticus* BD413, and *Acinetobacter* sp. SY-01 and shows over 45% aa identity per sequence pair. Recognition of an *Acinetobacter* subfamily was first proposed by Sullivan et al. [77] based upon deduced sequence comparisons of mature proteins and is strongly supported by the results shown in Fig. 1. Low sequence homologies internal to the latter two clades indicate significant divergence within these two groups. Within the *Acinetobacter* clade, divergence of BD413 protein from the closely related RAG-1/SY-01 group (89% aa identity) is evident.

Figure 2 shows the alignment of deduced aa sequences of lipases from subfamilies I.1 and I.2. Structural features previously identified in the family

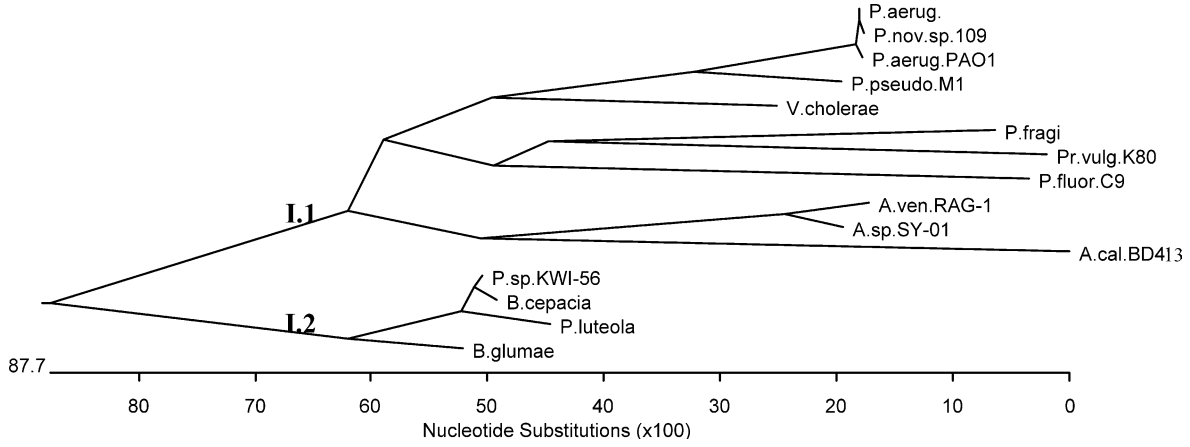


Fig. 1 Phylogenetic tree predicted from sequence alignment of prolipases from subfamilies I.1 and I.2. The rooted tree is derived by MEGALIGN from Lasergene sequence analysis software (DNASTAR, Madison, Wis.) with the following multiple sequence parameters: Clustal W method; gap penalty, 10; gap length penalty, 4. Sequence accession numbers and abbreviations: *Pseudomonas aeruginosa* (*P. aerug.*), D50587; *Pseudomonas* nov. sp. 109 (*P.* sp.109), P26877; *P. aeruginosa* PAO1 (*P. aerug.* PAO1), P26876; *Pseudomonas pseudoalcaligenes* —1 (*P. pseudo.* M1), A08195; *Vibrio cholerae* (*V. cholerae*), Y00557; *Pseudomonas fragi* IFO-12049, X14033; *Proteus vulgaris* (*Pr. vulg.* K80), U33845; *Pseudomonas fluorescens* C9 (*P. fluor.* C9), AF031226; *Acinetobacter venetianus* RAG-1, (*A. ven.* RAG-1), AF047691; *Acinetobacter* sp. SY-01 (*A. sp.* SY-01), AF518410; *Acinetobacter calcoaceticus* BD413 (*A. cal.* BD413), X80800; *Pseudomonas* sp. KWI-56 (*P.* sp. KWI-56), D10069; *Burkholderia cepacia*, M58494; *Pseudomonas luteola*, AF050153; *Burkholderia glumae* (*B. glumae*), AF70354

I.1 prototype lipase from *P. aeruginosa* (PAL)[61] and I.2 lipases represented by *B. glumae* (BGL) [64] provide a frame of reference for sequence comparisons. In describing the three-dimensional (3D) structure of PAL, Nardini et al. [61] noted strong similarities to homology family I.2 lipases (BGL) in core domains but reported the absence of an anti-parallel β-sheet following strand β7 (*P. aeruginosa* residues 226–243) and helix α10, found in BGL (*B. glumae* residues 307–310) and other family I.2 structures. Although a 3D structure of an *Acinetobacter* lipase has not yet been published, alignment results suggest *Acinetobacter* lipases lack these same topological features.

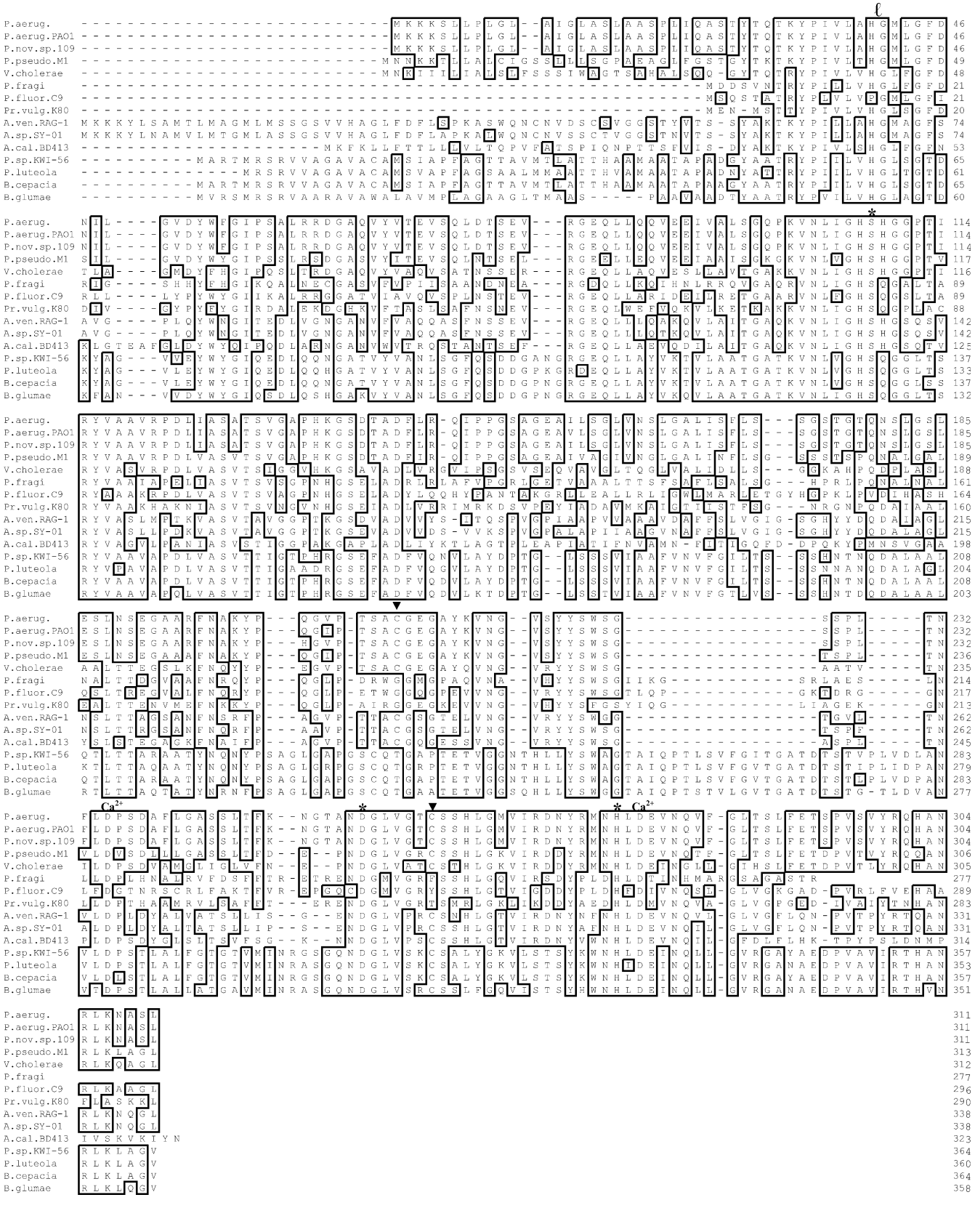
The strongly conserved sequences in both groups are those involved in enzyme stabilization and catalysis. Amino acid comparisons show that residues involved in Ca^{2+}-binding, disulfide bond formation, the catalytic Ser, Asp, and His residues, and the HG-dipeptide at the oxyanion hole (*P. aeruginosa* residues 40–41) are strongly conserved. Putative leader sequences, comprised of 20–26 hydrophobic residues, are also universally present. This finding is consistent with the requirement for Sec export to the periplasm [67]. Again, the strong sequence similarities among RAG-1, SY-01, and BD413 lipases support the proposal of an *Acinetobacter* lipase clade [77].

Fermentation and recovery

The distinctive biochemical properties of *Acinetobacter* lipases and microbial lipases in general must be considered in optimization of fermentation and recovery processes. Enzyme characteristics that may affect activity, stability, and yield under various fermentation conditions include strong affinity toward organic-aqueous interfaces, polymers, and solid adsorbents [51, 52, 58, 84], inactivation by various inhibitors or foaming [85], and susceptibility to proteolytic degradation [47, 58, 85]. Optimized lipase production is further complicated by choice of medium to include carbon source(s) [54], addition of inert compounds and hydrophobic adsorbents [51, 58, 73, 84], detergents and emulsifiers [45, 47, 54, 58, 73] or fermentation mode (batch, semicontinuous, or continuous) [51, 73, 85]. Low operational cost, high efficiency, process simplicity, environmental friendly productions are important considerations in commercial operations.

Martinez and Nudel [58] examined the effectiveness of several inert compounds on lipase secretion and stability in *A. calcoaceticus* whole cultures and cell-free supernatants. They found addition of gum arabic, glass beads, and Triton X-100 increased the release of lipase from cells 30–50% but only β-cyclodextrin and gum arabic maintained 100% lipase activity in cell-free extracts. They suggested cyclodextrin may function in sequestration of protease(s), thus preventing lipase degradation and increasing yield. Mahler et al. [54] examined the effects of carbon source (oleic acid, lactic acid) and its interaction with gum arabic in *A. calcoaceticus*. They reported a 2- to 5-fold increase in total lipase production in the presence of the polysaccharide. Gum arabic may increase lipase production by enhancing mechanical liberation of the enzyme at the surface of the cell but its removal may complicate downstream processing [54, 73, 86].

Acinetobacter radioresistens produces an alkaline lipase especially well-suited to detergent applications [17, 31]. It serves as a model enzyme for studies focusing on designing optimized scale-up, with emphasis on



improving production, separation, and recovery without significant increases in cost. Lipase production by this strain can be maximized when it is grown on *n*-hexadecane supplemented with olive oil [17]. Free fatty acids released from olive oil hydrolysis aid in emulsifying the hexadecane, thereby improving assimilation of the

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Fig. 2 Multiple sequence alignment of prolipases from subfamilies I.1 and I. 2. Alignment performed as in Fig. 1. Residues that match the consensus sequence (not shown) are *boxed*. Structural features previously identified in crystallized *P. aeruginosa* lipase (PAL) are labeled for comparison [61]. Symbols: * Catalytic triad residues, ▼ Cys residues involved in disulfide bridge formation, Ca^{2+} Asp residues involved in calcium binding, ℓ H-G dipeptide of the oxyanion loop

hydrocarbon. Moreover, association of fatty acids with hexadecane emulsions apparently reduces their inhibitory effect on lipase gene expression [17]. Further improvement in enzyme production and recovery from these carbon sources have been made by addition of hydrophobic polypropylene powders [52] that promote lipase adsorption and allow increased recovery in the centrifugation step. This culture method provided other advantages, in that it reduced foaming, provided higher volumetric lipase production, and decreased substrate consumption [52]. Increasing oxygen transfer by increasing the agitation speed enhanced both lipase production rate and maximum yield [16]. Furthermore, production increases of 130% were achieved in tank fermentors by attaching nylon fibers coated with hydrophobic acrylic resin to tank baffles [73]. Under these conditions, the lipase dissociates from the residual *n*-hexadecane and is adsorbed by the fabric. Recovery can be accomplished from the aqueous phase at 95% of total activity [73].

The differential affinity of *A. radioresistens* lipase for *n*-hexadecane-coated fabric with temperature was further explored as a low cost strategy to enhance recovery [84]. The lipase was adsorbed to hexadecane immobilized on fabric in batch cultures at 25°C and effectively desorbed from columns packed with fabric by lowering the temperature to 4°C. Production optimization strategies described for *A. radioresistens* may prove applicable to large-scale production of other bacterial lipases with similar hydrophobic properties and substrate utilization of the producing strain. Most importantly, these methods offer the potential for increased yields with very little additional expense.

Potential for industrial applications

The demand for enzymes in the United States alone is expected to surpass US $2.6 billion in 2004 and to grow 7% per annum through 2006 [25]. Lipases are forecast to be the fastest growing enzyme class, fueled by new applications in organic synthesis and pharmaceutical production, and by expanded penetration into the detergent industry [25]. The potential for many new lipase applications has driven a wide ranging search for novel enzymes [7, 57]. Studies of the structural basis of enantioselectivity [48], engineering enzyme specificity through directed evolution [8, 36], and improving technology to enhance production and yield [51, 52, 58] are also being pursued vigorously. The primary focus is on bacterial and fungal lipases because they are easier to produce, modify by recombinant DNA technology, and scale up for manufacturing applications. In this regard, lipases produced by *Pseudomonas* spp. are well described [26] and play a dominant role in industry. However, lipolytic strains of *Acinetobacter* have received increased attention as the search within the enzyme industry for novel biocatalysts accelerates. *Acinetobacter* spp. are also known for production of other potentially important commercial products, notably bioemulsifiers [20] and enzymes for bioremediation of hazardous wastes [1].

Interest in the biotechnological potential of lipases stems largely from their capacity to catalyze highly enantioselective biotransformations, both in aqueous and organic media. High enantiopreference is especially desired in the manufacture of pharmaceuticals or agrochemicals, when only one enantiomeric product (or intermediate) is biologically active [80]. Directed evolution has emerged as a key technology for enhancement of enzyme enantioselectivity and was first performed using lipase from *P. aeruginosa* [35, 39, 68]. The experimental strategy is to produce variant libraries of a wild-type gene using random mutagenesis (e.g., error-prone PCR, site saturation mutagenesis, DNA shuffling), overexpression of mutant genes in a suitable host, followed by screening for increased enantioselectivity [39]. The genes coding for enzymes demonstrating increased chiral selectivity are then used for a repeated round(s) of mutagenesis, expression, and screening. Using this strategy, the hydrolytic kinetic resolution of 2-methyldecanoic acid *p*-nitrophenyl ester was increased from 2% enantiomeric excess (ee) for wild-type *P. aeruginosa* lipase to 81% ee in a variant enzyme after only four rounds of mutagenesis [35, 39].

Although many enantioselective reactions have been described, large-scale industrial production of optically pure fine chemicals by lipase-catalyzed reactions is in its infancy, as evidenced by the relatively few examples that have been described [38, 82]. Recently, a novel enantioselective lipase (lipase A) from *Acinetobacter* sp. SY-01 has been described [30]. This lipase was shown to catalyze asymmetric hydrolysis of cis-(±)-2-(bromomethyl)-2-(2,4-dichlorophenyl)-1,3-dioxolane-4- methyl acetate, a racemic intermediate in the synthesis of the antifungal agent Itraconazole in an 81.5% conversion and 91.9% ee [30]. Production of the cis-(−)-isomer was significantly higher than for three commercial enzymes [30]. The enantioselectivity of other *Acinetobacter* lipases described to date remains to be documented, but such studies should yield results with significant industrial potential.

The list of compounds amenable to lipase-catalyzed biotransformations has continued to increase [70, 71, 82], but those with bulky substituents near the ester carbonyl group are notably absent. Mitsuhashi et al. [60] described such activity in a novel lipase purified from *Acinetobacter* nov. sp. KM109 and compared its hydrolytic activity against *p*-nitrophenyl esters and oleyl benzoate with that of commercial lipases. They found

four of ten commercial lipases also hydrolyzed *p*-nitrophenyl benzoate, but the greatest activity (*Candida cylindracea* lipase) was only 24% of the KM109 lipase. Furthermore, none of the commercial lipases hydrolyzed oleyl benzoate to any significant degree [60]. The potential of this enzyme for organic synthesis of similar sterically hindered compounds and its enantioselectivity remains to be explored.

Conclusions

Lipolytic strains of *Acinetobacter* have been isolated from many different environments and show high extracellular lipase activity when grown on an array of carbon substrates, many of which are amenable to high-yield recovery of the enzyme and downstream processing. These extracellular enzymes share many biochemical properties with other bacterial lipases currently used in such diverse applications as detergent manufacture, organic synthesis, oleo-chemistry, cosmetics production, and food processing. Expanded research on the biotechnological potential of these catalysts is justified, in light of the rapid expansion of enzyme manufacture and the ongoing search for novel enzymes with unique catalytic properties.

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