2018-12-07

Solvent stable microbial lipases: Current understanding and biotechnological applications

Barry Ryan
Technological University Dublin, barry.ryan@dit.ie

Priyanka Priyanka
Dublin Institute of Technology, D15127729@mydit.ie

Yeqi Tan
Dublin Institute of Technology, d11124994@mydit.ie

Gemma K Kinsella
Dublin Institute of Technology, gemma.kinsella@dit.ie

Gary T. Henehan
Dublin Institute of Technology, gary.henehan@dit.ie

Follow this and additional works at: https://arrow.dit.ie/schfsehart

Part of the Biochemistry, Biophysics, and Structural Biology Commons, Biotechnology Commons, and the Chemistry Commons

Recommended Citation

This Article is brought to you for free and open access by the School of Food Science and Environmental Health at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact yvonne.desmond@dit.ie, arrow.admin@dit.ie, brian.widdis@dit.ie.

This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 License
Solvent stable microbial lipases: Current understanding and biotechnological applications

Priyanka Priyanka*, Yeqi Tan*, Gemma K. Kinsella*, Gary T. Henehan*, Barry J. Ryan*♯

*Dublin Institute of Technology

♯ Corresponding Author (barry.ryan@dit.ie; Ph: 00353-1-4024379, Fax: +353 1 402 3000)

Acknowledgments
This work was supported by the Dublin Institute of Technology under the Fiosraigh Scholarship (PP and YT).
Abstract

Objective: This review examines our current understanding of microbial lipase solvent tolerance, with a specific focus on the molecular strategies employed to improve lipase stability in a non-aqueous environment.

Results: It provides an overview of known solvent tolerant lipases and of approaches to improving solvent stability such as; enhancing stabilising interactions, modification of residue flexibility and surface charge alteration. It shows that judicious selection of lipase source supplemented by appropriate enzyme stabilisation, can lead to a wide application spectrum for lipases.

Conclusion: Organic solvent stable lipases are, and will continue to be, versatile and adaptable biocatalytic workhorses commonly employed for industrial applications in the food, pharmaceutical and green manufacturing industries.

Keywords: industrial biocatalysis, lipase, lipase engineering, organic solvent stability, organic synthesis.

1. Introduction

The benefits of non-aqueous biocatalysis have strengthened the search for, and engineering of, solvent tolerant enzymes. Non-aqueous reactions can assist the dissolution of hydrophobic compounds, drive reaction equilibria from hydrolysis towards synthesis, have less undesirable side reactions (i.e. hydrolysis, polymerisation, racemisation) and generally are unhampered by microbial contamination (Kumar et al. 2016). For biotransformation processes in organic solvents to be more sustainable or “greener” than their chemical alternatives, they need to have higher productivity, better selectivity and should involve less steps for the synthesis of the desired product (Tao & Kazlauskas 2011, Wenda et al. 2011).

Lipases belong to the triacylglycerol ester hydrolase family (EC 3.1.1.3) and have long been of interest in non-aqueous synthesis (Sharma and Kanwar 2014). A major advantage of bacterial and fungal lipases, compared to plant and animal lipases, is their thermal and organic solvent tolerance. Microbial lipases are more widely used than yeast or fungal lipases since they are often more thermostable and offer higher catalytic activities (Salihu and Alam 2015).

This review will focus on the characteristics of lipases that contribute to their stability in organic solvents and on stability-enhancing modifications of lipases.
2. **Stability of lipases in organic solvents: structural features and interfacial activation**

The first lipase structures, from *Rhizomucor miehei* and human pancreas, were reported in the 1990s (Winkler et al. 1990; Derewenda et al. 1992). By October 2018, a search for ‘lipase structure’ in the RCSB Protein Data Bank (http://www.rcsb.org/pdb/) returned 273 hits. Structurally, all lipases fold in a similar fashion generating a characteristic α/β-hydrolase fold. This pattern contains parallel β-sheets surrounded by α-helices. This fold also gives rise to a catalytic triad composed of serine (Ser), histidine (His) and glutamate/aspartate (Glu/Asp) residues along with several oxyanion-stabilizing residues (Kazlauskas, 1994). This active site is conserved in most lipases irrespective of size (<1kDa to >60kDa).

A common characteristic of lipases is an increase in catalytic activity at a lipid water interface. This process, known as interfacial activation, involves a structural rearrangement of the lipase from an inactive to an active conformation. First identified in *M. miehei* lipase, the process is initiated by exposing a large hydrophobic area around the active site (Brady et al. 1990). The exposed hydrophobic area allows the anchoring of the lipase at the water/lipid interface thereby initiating catalysis. This resistance to denaturation at the water/lipid interface may account for lipase stability in organic solvents. The structural changes at the water/lipid interfaces typically involve a lid structure. The lid domain of lipases is amphipathic, having a hydrophobic and a hydrophilic side: in water the hydrophilic side of the lid faces the solvent while the hydrophobic side is directed towards the active site in a “closed” conformation. At the water/lipid interface the hydrophobic face becomes exposed allowing substrate access – the “open” conformation (Khan et al. 2017). This lid movement also changes the orientation of oxyanion-stabilizing residues to promote catalysis (Fischer et al. 2000). Lipase lid structures differ in terms of the position and number of the surface loops. Smaller lipases (e.g. *Rhizomucor miehei*) form the lid using a single α-helix while larger lipases (e.g. *Candida rugosa*) form the lid using two α-helices (see Figure One; Kazlauskas 1994b).

The activity of lipases in certain environments, e.g. in solvents, is dependent on the prevalence of the relevant conformation. Previous studies have shown that lid opening can be induced in an organic solvent (Maiangwa et al. 2017; Adlercreutz 2013). The efficiency of interfacial activation varies in different solvents (Abuin et al. 2007). Lipases show other changes in secondary structure in organic solvents. Lipase A from *Candida Antarctica*, for example, shows increased α-helical content following acetonitrile or acetone exposure and this change is
correlated with changes in hydrolytic activity (Yang et al. 2012). Significant research has focused on correlating the conformational changes of lipases in organic solvents with catalytic activity (Benkovic and Hammes-Schiffer 2003). The structural integrity of lipases in solvents has been probed by X-ray crystallography, circular dichroism (CD; Pelton and McLean 2000) and by nuclear magnetic resonance (NMR; Eppler et al. 2006; Kumar et al. 2014) often coupled with modelling and molecular dynamics simulation (YooPark et al. 2013). It is worth noting, however, that organic solvents can also cause denaturation and deactivation of lipases. Moreover, some solvents can compete for substrate binding and thus act as lipase inhibitors (Dror et al. 2015; Grosch et al. 2017).

Conversely, Zaks and Klibanov (1984) noted the importance of water for lipase activity through formation of non-covalent and hydrogen bonds with the enzyme. Water provides stability through a hydration shell, which protects it from direct contact with destabilising solvent (Díaz-García and Valencia-González 1995; Halling 1997). Loss of water molecules from the enzyme surface gives direct access to the solvent, thus disrupting its inter-, and intra-, molecular structure and provides rationale for solvent stable lipases (Schulze and Klibanov 1991; Dror et al. 2015).

3. Solvent Stable Lipase Sources

Many solvent stable lipases have been reported (see Table 1). For example, *Pseudomonas aeruginosa* AAU2 lipase is stable in organic solvents with a log P≥3.1 and even after 24 hours of incubation retains more than 70% of its activity (Bose and Keharia 2013). Similarly, a lipase from *Streptomyces* sp. CS133 was stable in 25% (v/v) *n*-Hexane (log P=3.5) and octane (log P=4.9; Mander et al. 2012) for 48 hours. In general, polar organic solvents are harsher on lipases than non-polar solvents. Polar organic solvents can cause enzyme deactivation by hydrogen bond disruption and by stripping the enzyme’s protective hydration shell. However, a few lipases, for example a lipase from *Bacillus sphaericus* MTCC 7542, were highly stable in both polar and non-polar organic solvents with a residual activity of 80-95% in all solvents even after 12-hr of incubation (Tamilarsan and Kumar 2012). Some lipases are stable in non-polar organic solvents (log P≥2), even after 7 days of incubation (e.g. *Stenotrophomonas maltophilia* CGMCC 4254 lipase). *Acinetobacter radioresistens* CMC-1 and *Acinetobacter* EH 28 lipases have higher stability and activity in 30% (v/v) *n*-Hexane, dimethyl sulfoxide (DMSO) and acetone than in 15% of the same solvents, indicating solvent activation (Ahmed et al. 2010). A lipase from *Burkholderia ambifaria* YCJ01 was noted to be stable for 60 days in a number of hydrophilic and hydrophobic solvents (25% v/v) and retained 100% activity in 25% (v/v) ethanol and 80% activity in 25% (v/v) acetonitrile, even after 30 days (Yao et al. 2013).
4. Improving organic solvent stability of lipases: random mutation and rational approaches

Exploiting our growing knowledge of lipase structure/function/stability relationships, in conjunction with protein engineering, can improve the catalytic properties of lipases in solvents (Villeneuve et al. 2000). A random mutation approach is useful when improving lipases when there is minimal structural information (Cobb et al. 2013). With the advent of computational modelling and molecular dynamic simulations, solvent tolerance enhancement from random mutation experiments can be understood at a structural level (Park, et al. 2013). In general, stabilising mutations may be categorised as: those based on increasing the stabilising interactions of surface residues, those reducing the flexibility of surface residues, and those changing the enzyme surface charge (see Table 2).

Enhanced stabilising interactions

The importance of stabilising interactions in protein stability has long been established as one of the ‘rules of thumb’ for protein engineering and the selection of target residues. Typical guiding principles include avoiding changes to residues that contribute to stabilising interactions or residues involved in the formation of secondary protein structure or those that might affect the formation of the active site (Yang et al. 2002). Based on the idea that the action of a solvent (stabilising or destabilising) on the protein can be defined by the balance between its preferential affinity for water or solvent (Timasheff 1993), many lipase engineering studies have involved mutation of surface residues.

For example, a lipase variant from Pseudomonas sp. KWI-56, which was found to be 40% more stable in 80% (v/v) DMSO than wild type, had only a single surface residue mutation (V304A; Nakano et al. 1998). Similarly, a G157R mutation in Pseudomonas aeruginosa LST-03 lipase introduced additional bonds that promoted salt bridge and H-bond formation. Another example showed that a single S194R mutation in Pseudomonas aeruginosa LST-03 could introduce new hydrogen bonds that resulted in the lipase being more stable in several solvents (Kawata and Ogino 2009, 2010). A methanol stable lipase variant from Proteus mirabilis was found to have mutations that introduced new side chain interactions (L64I) and novel H-bond formation (A70T and R33T). Additionally, G202E, K208N, G266S mutations are close to a Ca\(^{2+}\) binding site important for lipase stability. G266S introduced a new interaction with water and a residue that coordinates Ca\(^{2+}\) (Korman et al. 2013). A methanol stable variant of
Geobacillus stearothermophilus Lipase T6 (see Figure One) was found to have mutations that enabled the formation of hydrogen bonds with surface water (A269T, R374W) and a widened hydrogen bond network to enable more direct contact with the Zn$^{2+}$ coordinating residue (H86Y, Dror et al. 2014; Dror et al. 2015).

**Fig. 1** Methanol stable lipase variant H86Y/A269T/R374W from *Geobacillus stearothermophilus* T6 (PDB: 4X85) (Gihaz et al. 2018). The lid residues, F177-A192, are shown in yellow. The mutated residues are highlighted as H86Y (in cyan), A269T (in orange) and R374W (in purple). The image was generated using Pymol (DeLano 2018)

**Residue flexibility**

The flexibility of protein residues is often associated with enzyme stability (McAuley & Timson, 2016). It has been suggested that the stability of lipases is due to their conformation being more rigid in organic solvents (Sharma & Kanwar, 2014). This was supported by several studies including a DMSO-stable *Bacillus subtilis* lipase variant with an overall increased flexibility due to a single mutation to a more conformationally flexible residue (A269S), resulting in a less stable variant than the more rigid wild-type residue (Yedavalli & Madhusudhana Rao, 2013). The increase in rigidity associated with a network of intramolecular interactions, such as the extension of hydrogen bonds on the lipase surface, can prevent solvent penetration (Dror et al. 2014, 2015) and prevent protein denaturation (Reetz et al. 2006).
An increase in stabilising interactions has also been reported when targeting residue flexibility for improving *Candida antarctica* lipase B (CalB) stability in methanol (Park, et al. 2013). In this case, all the stabilising mutations were involved in the formation of additional hydrogen bonds with surface water (A8T, A92E, N97Q) or had shorter hydrogen bond distances (T245S). By contrast, mutations (e.g. T244D) that reduced the number of hydrogen bonds with water were linked to a reduced stability in methanol.

The flexibility of protein residues can be characterised using the B-factor value or Root Mean Square Deviation (RMSD) value. The B-factor, or the Debye Waller value, is used in crystallography to rate the flexibility of a residue in a protein structure: a higher value corresponds to greater flexibility (Reetz et al. 2006). This value represents the degree of elastic scattering caused by positional disorder, or the thermal motion, of an atom and hence a higher mobility of a protein residue is indicated by a high B-factor (Yuan et al. 2005). The selection of a residue based on its B-factor is known as a B-factor iterative test (B-FIT, Illanes et al. 2012) and is commonly used to guide enzyme thermostability enhancement (Wen et al. 2013; Kumar et al. 2014; Augustyniak et al. 2012). In this approach, residues with a high B-factor score were selected as the target for mutation. Using this approach, Reetz and colleagues (2010) successfully improved the stability of a lipase from *Bacillus subtilis* in polar organic solvents by mutating residues with the highest B factors to enhance the rigidity of the lipase.

Additionally, molecular dynamics simulations (MDS) are used to understand the dynamic nature of lipases in different environments. The relative flexibility of each individual residue in MDS is typically represented by Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) values. RMSD is a measure of deviation from the initial structure whereas RMSF is a measure of deviation from an average structure (Benson and Daggett 2012). Employing this approach, Park and colleagues (2013) rationalised the selection of residues with high RMSD as solvent affecting sites and the subsequent mutations (A8T, A92E, N97Q, T244D and T245S) enhanced CalB methanol stability and also had lower RMSD values in methanol.

Despite finding that greater lipase rigidity increases solvent stability, the decrease in enzyme flexibility observed in organic solvents is thought to be related to the corresponding decline in catalytic activity. The more rigid structure of LipA from *Candida antarctica* in ethyl esters is attributed to higher β-sheet content resulting in diminished catalytic activity (Yang et al. 2012). Yagonia and colleagues (2015) incorporated such considerations when improving CalB lipase stability and activity in methanol. By considering the catalytic orientation of CalB lipase, the structure was divided into a solvent affecting region and substrate-binding region for flexibility modulation based on B-factor and...
RMSD analysis. Mutations which increased rigidity in the solvent affecting region (A92E and T245S) showed higher methanol stability, but were less catalytically active, than the variants with activity enhancing mutations (V139E and A151D) and vice versa. The combination of both mutations resulted in mutants (V139E, A92E and V139E, T245S) that were more active and stable in presence of methanol (Yagonia et al. 2015).

### Surface charge and polarity

The effect of the polarity of organic solvent on surface charge has been correlated with lipase activity and stability (Iyer and Ananthanarayan 2008; Chakravorty et al. 2012; Jain and Mishra 2015). Solvents with low polarity cause the dispersal of enzyme hydrophobic domains due to solvent penetration, resulting in enzyme inactivation (Ogino and Ishikawa 2001). Conversely, the formation of a hydrated ion network, by charged amino acids, maintains the stability of lipases in organic solvents by preventing protein aggregation via repulsing electrostatic charges (Jain and Mishra 2015). In a set of random mutation studies by Kawata and colleagues (2009, 2010), *Pseudomonas aeruginosa* LST-03 lipase variants selected for stability in solvents were noted to possess mutations of surface residues (S164K, Y188F, L145H) that prevented penetration of the solvent into the protein. The increase in pI, due to the mutations (S164K, S211R, G157R, S194R, D209N, L145H), repulsed the basic organic solvent molecules through an ion repulsion interaction. In a comparable study, the mutation of lid residues to more hydrophobic residues (F146L, I289T) changed the accessibility of solvents to the active site of a lipase from *Pseudomonas sp. KWI-56* (Nakano et al. 1998).

Yedavalli and colleagues (2013) improved the stability of lipase (LipA) from *Bacillus substilis* in DMSO by modifying the loop secondary structure of the enzyme. In this case, the stable mutants had a similar secondary structure to the wild type, but with a more polar surface. Monsef Shokri and co-workers (2014) targeted a loop on the protein surface to improve the stability of *Pseudomonas sp.* lipase in non-aqueous solvent, by using the strategy of hydrophobic residue substitution (Arnold 1990). The variants that were more stable in hydrophilic organic solvents had hydrophobic mutations at position 219 (N219A, N219I, N219L), resulting in increased lipase rigidity.

TABLE 2 HERE
5. Selected applications of Organic Solvent Stable Lipases by industry

Solvent compatible lipases are in use in the food, (bio)pharmaceutical and environmental industries (Ahmed et al. 2010). Selected applications in these industries are discussed below.

Food industry; Flavours and Fragrances

The world flavour and fragrance market was $22 billion (USD) in 2011 and has been increasing at a rate of 5.6% annually (Badgujar et al. 2016). Although the isolation and extraction of flavour esters from natural sources is expensive, consumers prefer products with a ‘natural’ label (Ahmed et al. 2010). Therefore, alternatives to chemical synthesis of flavour esters, including alternative production technologies such as esterification by solvent stable lipase, have gained attention (Matte et al. 2016 and see Table 3).

Pharmaceutical Industry: Regio- and Stereo-selective Resolution

Lipases are widely used for the kinetic resolution of compounds (Xun et al. 2013). The regioselectivity of lipases has been exploited for the resolution of racemic alcohols and kinetic resolution of racemic mixtures of compounds such as flurbiprofen (C. antarctica lipase Novozyme® 435) and N-hydroxymethyl vince lactam (Mucor meihei lipase; Xun et al. 2013). Interestingly, lipase resolution has also been explored in the production of herbicides (phenoxypropionate) by the resolution of 2-halopropionic acids and esterification of (S)-isomers in butanol and hexane (Hasan et al. 2006).

Lipase regioselectivity has been used for the synthesis of compounds that are difficult to synthesize by chemical methods (Miyazawa et al. 2014). Non-ionic and biodegradable sugar esters have extensive applications in detergents, pharmaceutical and oral care products. Their conventional production, involving chemical sugar and fatty acid esterification, is difficult due to poor regioselectivity and the low organic solvent solubility of sugars. This esterification was achieved by immobilized lipase B from Candida Antarctica (Novozyme® 435) in DMSO and acetone (1:10 v/v). This dual solvent environment has been used for the production of a xylene caproate ester with 64% yield (Abdulmalek et al. 2016). Various other precursor molecules required for the manufacturing of a range of pharmaceutical and agrochemical products are currently synthesised by lipases in solvents (see Table 4).
In recent times, much attention has focused on polymer research due to their increased use in biomedical research, food packaging and agricultural industries. However, the disposal of these polymers is a critical environmental issue, and has led to the development of biodegradable polymers (e.g. polyesters) as an alternative to traditional plastics (Banerjee et al. 2014). Solvent stable lipases have been widely used as catalysts for the synthesis of such biodegradable polyesters (Barrera-rivera & Flores-carreón 2012 and see Table 5). The lipase from Candida antarctica lipase B (CalB) is the most common catalyst used for polyester synthesis (Chen et al. 2008).

The production of biodegradable polyesters plays a significant role in a green environmental approach to packaging; however, recycling is equally important from an environmental perspective. A wide range of aromatic and aliphatic polyesters including poly(ethylene terephthalate), poly(butylene succinate), poly (β-caprolactone) and poly(lactic) acids are used in the production of medical biomaterials such as surgical sutures and reinforcing plates (Kobayashi, 2010). Chemical recycling has limited applications due to its high-energy demand, both in terms of temperature and pressure. However, degradation of biopolymers by enzymatic processes can occur with a lower energy requirement and in milder conditions (see Table 5 for relevant examples). Moreover, chemical based recycling cleaves polymers randomly, generating varying molecular weight oligomers while lipase catalyzed degradation involves cleaving the amorphous regions of a polymer first, followed by its crystalline regions, consistently resulting in oligomers with lower molecular weights (Banerjee et al. 2014). The lipase from Candida antarctica has been successfully utilised to degrade poly(β-caprolactone; PCL) in dry toluene at 60°C. This ‘one-pot degradation-polymerisation’ reaction successfully recycled poly(β-caprolactone; Kobayashi et al. 2000). Although lipases can hydrolyse poly(β-caprolactone) in aqueous solution; the low solubility of hydrophobic PCL in water means that, solvents are generally used for their degradation (Aris et al. 2016).
6. Considerations in the development of organic solvent stable lipases for industrial application

Currently, industrial biocatalysis requires lipases for existing and emerging industries. New lipases can be delivered via biodiscovery or through improving existing lipase properties by protein engineering. Despite the increasing number and availability of organic solvent stable lipases there is a lack of translation of lab scale biocatalysis to industry scale. Optimising an enzyme for use in a given application is challenging as it is difficult to make the biocatalytic operational space (e.g. temperature, pH, pressure etc.) as wide as the chemical counterpart. Enzymes are prone to denaturation and deactivation under extreme processing conditions (Tufvesson et al. 2013; Ringborg and Woodley 2016). In reactions involving organic solvents there are several thermodynamic constraints including; interaction with the enzyme, substrate solubility and enzyme solubility (Grosch et al. 2017). This results in the need to understand the effective concentration of substrate available to the enzyme, competitive inhibition by the organic solvent, transition state stabilisation (Dutta Banik et al. 2016), as well as steric effects (Wang et al. 2016). In the past, biodiscovery and engineering of existing organic solvent lipases commenced without a defined target reaction; however, now these thermodynamic constraints are key drivers in the enzyme selection procedure for industrial scale biocatalysis (Ringborg and Woodley 2016). It is prudent to identify the organic solvent effects; such as solvation of substrate, inhibition by solvent molecule, and water activity, on catalysis (Sandoval et al. 2001; Kulschewski et al. 2013; Grosch et al. 2017).

7. Conclusion and Future Directions

Lipases continue to be an important biocatalyst in the food, pharmaceutical, and chemical industries (De Godoy Daiha et al. 2015). Previous lipase engineering efforts have demonstrated that, through engineering approaches, organic solvent stability can be achieved. The utility of lipases in biocatalysis depends on finding a balance between enzyme rigidity and catalytic activity for many applications. A recent study looked at filling lipase solvent tunnels with aromatic interactions to improve lipase stability in methanol, with the results demonstrating a stabilisation of 81-fold compared with wild-type (Gihaz et al. 2018). This rational approach could be extended to other lipases for stabilization in organic solvents.

The use of novel solvent systems such as ionic liquids or deep eutectic solvents appears to offer promising alternatives to traditional organic solvents. Recently, Brogan and colleagues (2018) reported that the combination of chemical modification and ionic liquids produced a highly robust glucosidase that displayed “solvent induced
substrate promiscuity” and activity at temperatures up to 137°C. Thus, through a synergistic combination of enzyme modification and solvent choice the biocatalytic capability of enzymes was enhanced. It would be of interest to explore whether such chemical modification might lead to similar stabilisation of lipases in solvents. It is clear that the drive for more sustainable catalysis will provide an impetus for this field in the coming years.

8. Compliance with Ethical Standards

Funding: This work was supported by the Dublin Institute of Technology under the Fiosraigh Scholarship (PP and YT).

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Authorship: Conceived study (PP, YT, GKK, GTH, BJR), Performed research (PP, YT), Analyzed data (PP, YT), Contributed methods (GKK, GTH, BJR), Wrote the paper (PP, YT, GKK, GTH, BJR).

Data Availability: The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

References:


Timasheff SN (1993) The Control of Protein Stability and Association by Weak Interactions with Water: How Do


YooPark HJ, Park K, Je Y (2013) Understanding the effect of tert-butanol on Candida antarctica lipase B using


doi: 10.1126/science.6729453
<table>
<thead>
<tr>
<th>Lipase Source</th>
<th>Stability test condition</th>
<th>Solvent % (v/v)</th>
<th>Activity</th>
<th>Solvent System</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudoalteromonas lipolytica</em> SCSIO 04301</td>
<td>12 hours at room temperature</td>
<td>50%</td>
<td>&gt;100% residual activity</td>
<td>ethanol, acetone, DMSO, t-butanol, hexane</td>
<td>(Su et al. 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;50% residual activity</td>
<td>acetonitrile, t-butanol, toluene, hexane</td>
<td></td>
</tr>
<tr>
<td><em>Penicillium corylophilum</em></td>
<td>37°C after 1 hour</td>
<td></td>
<td>&gt;100% residual activity</td>
<td>ethanol, acetone</td>
<td>(Romero et al. 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;50% residual activity</td>
<td>methanol, butanol and hexanol</td>
<td></td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. OC119-7</td>
<td>30°C for 24 hours</td>
<td></td>
<td>&gt;70% residual activity</td>
<td>methanol, ethanol, acetone</td>
<td>(Ayaz et al. 2014)</td>
</tr>
<tr>
<td>Organism</td>
<td>Condition</td>
<td>Activity</td>
<td>Solvents</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>---------------------------</td>
<td>----------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Idiomarina</em> sp. W33</td>
<td>30°C for 12 days</td>
<td>&gt;70% residual</td>
<td>toluene, cyclohexane, n-hexane, 1-decanol and isoctane</td>
<td>(Li et al. 2014)</td>
<td></td>
</tr>
<tr>
<td><em>Haloarcula</em> sp. IG41</td>
<td>30°C for 2 and 5 days</td>
<td>&gt;70% residual activity</td>
<td>toluene, cyclohexane, n-hexane, 1-decanol and isoctane</td>
<td>(Li &amp; Yu 2014)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;60% residual</td>
<td>chloroform and n-hexane</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ALA1</td>
<td>30 min at 37°C</td>
<td>&gt;90% residual activity</td>
<td>acetone, benzene, ethanol, methanol, 2-propanol and toluene</td>
<td>(Ben Bacha et al. 2016)</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>30 min at 37°C</td>
<td>25% &gt;80% relative activity</td>
<td>acetone, t-butanol</td>
<td>(Sivaramakrishnan &amp; Incharoensa kdi 2016)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>~100% relative activity</td>
<td>methanol and ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>30 min at room</td>
<td>&gt;120% residual</td>
<td>Diethyl ether, DMSO</td>
<td>(Kamarudin)</td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus epidermidis</strong> AT2</td>
<td>Temperature</td>
<td>Activity</td>
<td>Solvents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------</td>
<td>----------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>~100% residual activity</td>
<td>n-hexane, toluene, acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Burkholderia cepacia</strong> RQ3</th>
<th>Temperature</th>
<th>Activity</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°C for several days</td>
<td>half-life &gt;10 days</td>
<td>isopropanol, ethanol and n-octane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>half-life of 6 days</td>
<td>DMSO</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Aneurinibacillus thermoaerophilus</strong></th>
<th>Temperature</th>
<th>Activity</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min of incubation</td>
<td>&gt;60% relative activity</td>
<td>propyl acetate, p-xylene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;80% relative activity</td>
<td>toluene, benzene, 1-propanol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;100% relative activity</td>
<td>DMSO and methanol</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Xanthomonas oryzae</strong></th>
<th>Temperature</th>
<th>Activity</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>70°C for 24 hours</td>
<td>20% Specific activity of &gt;250 U/mg</td>
<td>heptane, hexane, methanol</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Aureobasidium</strong></th>
<th>Temperature</th>
<th>Activity</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min at 37°C</td>
<td>10% &gt;80% relative activity</td>
<td>methanol, acetonitrile, ethanol and</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>melanogenum</strong></th>
<th>followed by 24 hours at 4°C</th>
<th></th>
<th>chloroform</th>
<th>apaiboon et al. 2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pseudomonas sp. DMVR46</strong></td>
<td>37°C for 4 hours</td>
<td>5%</td>
<td>&gt;30% residual activity</td>
<td>ethanol, isopropanol, acetone</td>
</tr>
</tbody>
</table>
Table 2 Lipase engineering towards improved organic solvent stability. Studies are categorised based on type of mutation: random, surface or flexible residues. The lipase, its mutation(s), the solvent system (and logP value) are shown.

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Mutation(s)</th>
<th>Organic solvent (logP)</th>
<th>Wild type activity (Incubation time/half life)</th>
<th>Mutant activity (Incubation time)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Random mutation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas sp. KWI-56</em></td>
<td>S26G, F146L, I289T, and V304A</td>
<td>DMSO (logP = -1.35)</td>
<td>60% residual activity (120min)</td>
<td>90% residual activity, (120min)</td>
<td>(Nakano et al. 1998)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa LST-03</em></td>
<td>S164K, T188F, S211R</td>
<td>Cyclohexane (logP = 3.44)</td>
<td>6.0 days half life</td>
<td>54.8 days half life</td>
<td>(Kawata and Ogino 2009)</td>
</tr>
<tr>
<td></td>
<td>S155L, G157R, G177V, S194R, S202W, D209N</td>
<td>Cyclohexane (logP = 3.44)</td>
<td></td>
<td>41.1 days half life</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa LST-03</em></td>
<td>S155L</td>
<td>n-Octane (logP = 5.15)</td>
<td>6.6 days half life</td>
<td>31.6 days half life</td>
<td>(Kawata and Ogino 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>6.9 days half life</td>
<td>17.4 days half life</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n-Heptane (logP = 4.5)</td>
<td>9.5 days half life</td>
<td>&gt;100 days half life</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Compound</td>
<td>logP</td>
<td>S164K Half-life</td>
<td>S164K Half-life</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
<td>-------</td>
<td>----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Hexane</td>
<td>3.44</td>
<td>13.0 days</td>
<td>28.0 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclohexane</td>
<td>3.44</td>
<td>8.0 days</td>
<td>15.8 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>-1.35</td>
<td>6.9 days</td>
<td>14.3 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Decane</td>
<td>6.25</td>
<td>8.9 days</td>
<td>&gt;100 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Octane</td>
<td>5.15</td>
<td>6.6 days</td>
<td>&gt;100 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Hexane</td>
<td>3.44</td>
<td>13.0 days</td>
<td>&gt;100 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclohexane</td>
<td>4.00</td>
<td>6.0 days</td>
<td>44.3 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Heptane</td>
<td>4.5</td>
<td>9.5 days</td>
<td>33.5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Decane</td>
<td>6.25</td>
<td>8.9 days</td>
<td>&gt;100 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Octane</td>
<td>5.15</td>
<td>6.6 days</td>
<td>37.4 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Hexane</td>
<td>3.44</td>
<td>13.0 days</td>
<td>&gt;100 days</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Lipase</td>
<td>Incubation</td>
<td>Half-Life 1</td>
<td>Half-Life 2</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>G157R</td>
<td>Cyclohexane (logP = 4.00)</td>
<td>6.0 days</td>
<td>&gt;100 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Heptane (logP = 4.5)</td>
<td>9.5 days</td>
<td>&gt;100 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene (logP = 2.73)</td>
<td>26.6 days</td>
<td>&gt;100 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Decane (logP = 6.25)</td>
<td>8.9 days</td>
<td>16.4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Octane (logP = 5.15)</td>
<td>6.6 days</td>
<td>25.4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Hexane (logP = 3.44)</td>
<td>13.0 days</td>
<td>28.8 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclohexane (logP = 4.00)</td>
<td>6.0 days</td>
<td>21.0 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-Heptane (logP = 4.5)</td>
<td>9.5 days</td>
<td>10.2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S211R</td>
<td>n-Octane (logP = 5.15)</td>
<td>6.6 days</td>
<td>18.7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Methanol (logP = -0.74)</td>
<td>Inactivated</td>
<td>80% residual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td>(16 hrs)</td>
<td>activity (16 hrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Korman et al. 2013)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface residue properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Candida antarctica</strong> lipase B (CalB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N97Q</td>
<td>Methanol (logP = -0.74)</td>
<td>~30% residual activity (72 hrs)</td>
<td>~50% residual activity (72 hrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N264Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D265E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D223E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N292Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong> lipase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I12L, W42L, A68S, P119S, L140F and Y139K</td>
<td>DMSO (logP = -1.35)</td>
<td>100% relative activity (5 min)</td>
<td>300% relative activity (5 min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Park et al. 2012)
(Yedavalli and Madhusudhana Rao 2013)
<table>
<thead>
<tr>
<th>Pseudomonas sp. lipases</th>
<th>N219A</th>
<th>DMF (logP = -1.51)</th>
<th>~25% residual activity (12 min)</th>
<th>~50% residual activity (12 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N219I</td>
<td></td>
<td>Methanol (logP = -0.74)</td>
<td>~10% residual activity (100 min)</td>
<td>~40% residual activity (100 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol (logP = -0.24)</td>
<td>~10% residual activity (10 min)</td>
<td>~40% residual activity (10 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n-Propanol (logP = 0.25)</td>
<td>Inactivated (5 min)</td>
<td>~30% residual activity (5 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMF (logP = -1.51)</td>
<td>~25% residual activity (12 min)</td>
<td>~60% residual activity (12 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanol (logP = -0.74)</td>
<td>~10% residual activity (100 min)</td>
<td>~60% residual activity (100 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol (logP = -0.24)</td>
<td>~10% residual activity (10 min)</td>
<td>~50% residual activity (10 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n-Propanol (logP = 0.25)</td>
<td>Inactivated (5 min)</td>
<td>~30% residual activity (5 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMF (logP = -1.51)</td>
<td>~25% residual activity (12 min)</td>
<td>~60% residual activity (12 min)</td>
</tr>
<tr>
<td>N219L</td>
<td></td>
<td>Methanol (logP = -0.74)</td>
<td>~10% residual activity (10 min)</td>
<td>~60% residual activity (10 min)</td>
</tr>
</tbody>
</table>

(Monsef Shokri et al. 2014)
<table>
<thead>
<tr>
<th>Protein / Solvent</th>
<th>Activity</th>
<th>Half Life</th>
<th>Half Life</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geobacillus stearothermophilus T6 Lipase</strong></td>
<td>Ethanol (logP = -0.24)</td>
<td>~10% residual activity (10 min)</td>
<td>~60% residual activity (10 min)</td>
</tr>
<tr>
<td>A269T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q185L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H86Y/A269T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q185L/ A269T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H86Y/A269T/ R374W</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanol (logP = -0.74)</strong></td>
<td>2.9 min half life</td>
<td>77 min half life</td>
<td>77 min half life</td>
</tr>
<tr>
<td><strong>n-Propanol (logP = 0.25)</strong></td>
<td>Inactivated (5 min)</td>
<td>~60% residual activity (5 min)</td>
<td>~60% residual activity (5 min)</td>
</tr>
<tr>
<td><strong>Residue flexibility</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacillus subtilis Lipase</strong></td>
<td>Acetonitrile (logP = -0.34)</td>
<td>&lt;30 mins half life</td>
<td>&lt;300 mins half life</td>
</tr>
<tr>
<td>M134D/I157M</td>
<td>Acetonitrile (logP = -0.34)</td>
<td>&lt;30 mins half life</td>
<td>&gt;300 mins half life</td>
</tr>
<tr>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>&lt;10 hrs half life</td>
<td>&lt;25 hrs half life</td>
</tr>
<tr>
<td></td>
<td>DMF (logP=-1.51)</td>
<td>&lt;10 hrs half life</td>
<td>&lt;25 hrs half life</td>
</tr>
<tr>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>&lt;10 hrs half life</td>
<td>&lt;50 hrs half life</td>
</tr>
<tr>
<td></td>
<td>DMF (logP=-1.51)</td>
<td>&lt;10 hrs half life</td>
<td>&lt;25 hrs half life</td>
</tr>
<tr>
<td></td>
<td>M134D/I157M/ Y139C</td>
<td>Acetonitrile (logP = -0.34)</td>
<td>&lt;30 mins half life</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>&lt;10 hrs half life</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMF (logP = -1.51)</td>
<td>&lt;10 hrs half life</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>M134D/I157M/ Y139C/K112D</th>
<th>Acetonitrile (logP = -0.34)</th>
<th>&lt;30 mins half life</th>
<th>&lt;900 mins half life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>&lt;10 hrs half life</td>
<td>&gt;100 hrs half life</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMF (logP = -1.51)</td>
<td>&lt;10 hrs half life</td>
<td>&gt;100 hrs half life</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>M134D/I157M/Y139C/K112D/R33G</th>
<th>Acetonitrile (logP = -0.34)</th>
<th>&lt;30 mins half life</th>
<th>&gt;1500 mins half life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DMSO (logP = -1.35)</td>
<td>&lt;10 hrs half life</td>
<td>&gt;200 hrs half life</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMF (logP = -1.51)</td>
<td>&lt;10 hrs half life</td>
<td>&lt;200 hrs half life</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Candida antarctica</strong> lipase B (CalB)</th>
<th>A8T</th>
<th>Methanol (logP = -0.74)</th>
<th>35 hrs half life</th>
<th>52 hrs half life (Park et al. 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A92E</td>
<td></td>
<td></td>
<td>63 hrs half life</td>
</tr>
<tr>
<td></td>
<td>N97Q</td>
<td></td>
<td></td>
<td>52 hrs half life</td>
</tr>
<tr>
<td></td>
<td>T244D</td>
<td></td>
<td></td>
<td>59 hrs half life</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Candida antarctica</strong> lipase B (CalB)</th>
<th>V139E</th>
<th>Methanol (logP = -0.74)</th>
<th>~20% residual activity (24 hrs)</th>
<th>~50% residual activity (24 hrs) (Yagonia et al. 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A151D</td>
<td></td>
<td>~30% residual activity (24 hrs)</td>
<td>~50% residual</td>
</tr>
<tr>
<td></td>
<td>A92E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>activity (24 hrs)</td>
<td>~50% residual activity (24 hrs)</td>
<td>~60% residual activity (24 hrs)</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td></td>
</tr>
<tr>
<td>T245S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V139E, A92E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V139E, T245S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Lipases commonly used in the food industry for the production of food flavours and aromas. The table shows lipase source, the product of the lipase catalysed reaction along with the solvent system employed for synthesis and typical yields.

<table>
<thead>
<tr>
<th>Lipase Source</th>
<th>Product (application)</th>
<th>Lipase state</th>
<th>Yield</th>
<th>Solvent system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus oryzae</em></td>
<td>Cis-3-hexen-1-yl-acetate (fresh/floral odour)</td>
<td>In dry mycelium</td>
<td>98%</td>
<td>60mM acetic acid and cis-3-hexen-1-ol</td>
<td>Kirdi et al. 2017</td>
</tr>
<tr>
<td><em>Pseudomonas cepacia</em></td>
<td>Cinnamyl Propionate (spicy floral flavour)</td>
<td>Immobilized on hydroxylpropyl methyl cellulose and polyvinyl alcohol</td>
<td>&gt;90%</td>
<td>Cinnamyl alcohol: vinyl propionate (1:2) with 1ml n-hexane or toluene (non-polar solvents)</td>
<td>Badgujar et al. 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;50%, &lt;80%</td>
<td>Cinnamyl alcohol: vinyl propionate (1:2) with 1ml acetone, dioxane (hydrophobic)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Lipase Source</strong></th>
<th><strong>Product (application)</strong></th>
<th><strong>Lipase state</strong></th>
<th><strong>Yield</strong></th>
<th><strong>Solvent system</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus oryzae</em></td>
<td>Cis-3-hexen-1-yl-acetate (fresh/floral odour)</td>
<td>In dry mycelium</td>
<td>98%</td>
<td>60mM acetic acid and cis-3-hexen-1-ol</td>
<td>Kirdi et al. 2017</td>
</tr>
<tr>
<td><em>Pseudomonas cepacia</em></td>
<td>Cinnamyl Propionate (spicy floral flavour)</td>
<td>Immobilized on hydroxylpropyl methyl cellulose and polyvinyl alcohol</td>
<td>&gt;90%</td>
<td>Cinnamyl alcohol: vinyl propionate (1:2) with 1ml n-hexane or toluene (non-polar solvents)</td>
<td>Badgujar et al. 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;50%, &lt;80%</td>
<td>Cinnamyl alcohol: vinyl propionate (1:2) with 1ml acetone, dioxane (hydrophobic)</td>
<td></td>
</tr>
<tr>
<td><strong>Organism</strong></td>
<td><strong>Enzyme/Compound</strong></td>
<td><strong>Immobilisation</strong></td>
<td><strong>Solvent</strong></td>
<td><strong>Reference</strong></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>-------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus aerius</em></td>
<td>Isoamyl acetate (pear/banana flavour)</td>
<td>Immobilised on silica gel matrix</td>
<td>1:1 ratio of acetic acid and isoamyl alcohol</td>
<td>Narwal et al. 2016</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>Ethyl lactate (fruity odour and fruity flavour)</td>
<td>Immobilized on magnetite particles</td>
<td>(1:1) ethyl alcohol and lactic acid</td>
<td>Jain &amp; Mishra, 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isobutyl acetate (pineapple flavour)</td>
<td></td>
<td>Isobutyl alcohol and acetic acid (1:1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida rugosa</em></td>
<td>Ethyl caprylate (flavour ester)</td>
<td>Immobilised on exfoliated graphene oxide</td>
<td>Ethanol and cyclo-octane (0.15:0.1M)</td>
<td>Patel et al. 2015</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>Ethyl</td>
<td>Immobilised</td>
<td>Ethanol/butyric</td>
<td>Vrutika &amp;</td>
<td></td>
</tr>
<tr>
<td>Microorganism</td>
<td>Product and Characteristics</td>
<td>Reaction Conditions</td>
<td>Product and Yield (%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------</td>
<td>--------------------------------------------</td>
<td>------------------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. DMVR46</td>
<td>Butyrate (tropical fruit flavour)</td>
<td>Immobilisation on multi-walled carbon nanotubes</td>
<td>Acid and n-heptane (0.15:0.2M)</td>
<td>Datta, 2015</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. DMVR46</td>
<td>Pentyl valerate (fruity aroma)</td>
<td>Immobilisation into AOT-organogels</td>
<td>88%</td>
<td>Vrutika et al. 2014</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus safensis</em></td>
<td>Ethyl laurate (waxy odour and flavour)</td>
<td>Purified enzyme</td>
<td>Lauric acid and ethanol (1:1)</td>
<td>Kumar et al. 2014</td>
<td></td>
</tr>
</tbody>
</table>
**Table 4** Pharmaceutical products synthesized by lipases in a solvent system. The table shows the product formed through the lipase catalysed reaction as well as its application, the lipase involved and the mode of operation including the solvent system.

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Lipase</th>
<th>Lipase state</th>
<th>Solvent system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-Indano</td>
<td>Precursor of drugs (Sertraline, Indinavir, Irindalone, Rasagiline mesilate)</td>
<td><em>Candida antarctica</em></td>
<td>Immobilized on Cashew apple bagasse support</td>
<td>Toluene and diisopropyl ether</td>
<td>De Souza et al. 2016</td>
</tr>
<tr>
<td>Sugar Fatty Acid Ester</td>
<td>6-O-glucose tetradecanoate</td>
<td><em>Candida antarctica</em></td>
<td>Immobilized lipase Novozym435</td>
<td>Dimethylformamide</td>
<td>Degn et al. 1999</td>
</tr>
<tr>
<td></td>
<td>6-O-glucose octadecanoate</td>
<td><em>Mucor mehei</em></td>
<td>Immobilized from NOVO industries</td>
<td>Heptane</td>
<td>Oguntimein et al. 1993</td>
</tr>
<tr>
<td></td>
<td>6-O-acetyl glucopyranoside</td>
<td>Porcine pancreatic</td>
<td>Free enzyme</td>
<td>Hexane</td>
<td>Sharma &amp; Chattopadhyay 1993</td>
</tr>
<tr>
<td></td>
<td>Dilauroyl maltose</td>
<td><em>Candida antarctica</em></td>
<td>Immobilized by Novo-Nordisk</td>
<td>Acetone and n-hexane</td>
<td>Jia et al. 2010</td>
</tr>
<tr>
<td>Compound</td>
<td>Enzyme</td>
<td>Immobilization Method</td>
<td>Solvent</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------</td>
<td>------------------------------------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>Fructose Oleate</td>
<td>Thermomyces lanuginosus and Pseudomonas fluorescens</td>
<td>Immobilized on functionalized silica</td>
<td>t-butyl alcohol</td>
<td>Vescovi et al. 2017</td>
<td></td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Cholesterol reduction, anti-viral and anti-inflammatory</td>
<td>Candida rugosa</td>
<td>n-Hexane</td>
<td>Jiang et al. 2013</td>
<td></td>
</tr>
<tr>
<td>(R,S)-1-phenylethanol</td>
<td>cosmetics and the pharmaceutical industry</td>
<td>Pseudomonas stutzeri</td>
<td>n-hexane</td>
<td>Cao et al. 2012</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2: Continued.
<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Lipase</th>
<th>Lipase state</th>
<th>Solvent system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiral mandelic acid and its derivates</td>
<td>Intermediates for pharmaceutical industry</td>
<td><em>Burkholderia ambifaria</em></td>
<td>Free enzyme</td>
<td>Diisopropyl ether</td>
<td>Yao et al. 2013</td>
</tr>
<tr>
<td>Ethyl oleate</td>
<td>Solvent for preparation of steroids; plasticizer</td>
<td><em>Geobacillus stearothermophilus</em></td>
<td>Immobilized on cellulosic nanogel</td>
<td>Ethanol and DMSO</td>
<td>Kumar et al. 2015</td>
</tr>
<tr>
<td>β-sitostanol ester</td>
<td>Decreasing cholesterol absorption</td>
<td><em>Ophiostoma piceae</em></td>
<td>Crude enzyme</td>
<td>Isooctane</td>
<td>Molina-Gutiérrez et al. 2016</td>
</tr>
<tr>
<td>Leutin Dipalmitate</td>
<td>Inhibition of Age-related macular degeneration (AMD); For high acuity vision; decrease UV-induced damage on skin</td>
<td><em>Candida antarctica</em></td>
<td>Immobilized on macroporous acrylic resin</td>
<td>Toluene</td>
<td>Wang et al. 2015</td>
</tr>
</tbody>
</table>
**Table 5** Examples of the production and degradation of polymers by lipases. The source of the lipase, the state utilised and the substrate and solvent system employed are shown.

<table>
<thead>
<tr>
<th>Action</th>
<th>Lipase Source</th>
<th>Lipase state</th>
<th>Substrate</th>
<th>Solvent system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester synthesis</td>
<td><em>Yarrowia lipolytica</em></td>
<td>Immobilized on microporous resin</td>
<td>β-caprolactone</td>
<td>Heptane</td>
<td>Barrera-rivera &amp; Flores-carreón 2012</td>
</tr>
<tr>
<td></td>
<td><em>Candida antarctica</em></td>
<td>Immobilized on nanoclays</td>
<td>β-caprolactone</td>
<td>Dry toluene</td>
<td>Öztürk Düşkınkur et al. 2014</td>
</tr>
<tr>
<td></td>
<td><em>Candida antarctica</em></td>
<td>Immobilized from Novozyme</td>
<td>β-caprolactone and β-thiocaprolactone</td>
<td>Toluene</td>
<td>Duchiron et al. 2017</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em></td>
<td>Free</td>
<td>Polyhydroxyalkano</td>
<td>Chloroform</td>
<td>Kanmani</td>
</tr>
<tr>
<td>microorganism</td>
<td>enzyme type</td>
<td>polymer type</td>
<td>solvent</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td><em>subtilis</em></td>
<td>enzyme</td>
<td>ates (PHAs)</td>
<td>m</td>
<td>et al. 2016</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus us</em></td>
<td>Free enzyme</td>
<td>Poly(β-caprolactone)</td>
<td>Chloroform</td>
<td>Khan et al. 2017</td>
<td></td>
</tr>
<tr>
<td><em>plantarum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>