



Microbial lipases: An overview of screening, production and purification

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ABSTRACT

Lipases are industrially important biocatalyst, particularly microbial lipases and therefore, screening, production and purification of lipase enzyme from microbial strains are continuously emerging to fulfil the needs of pharmaceutical and food industries. More recently, various cost effective and efficient approaches are being attempted to increase the production of lipases in microbial strains. In this junction, this article attempt to connect production of lipases using various microbial strains such as bacteria, fungi and yeast has been highlighted and discussed, in order to enable researchers to choose an appropriate strains for lipase production. Then, screening of lipase producer and production of lipases under different fermentation system was discussed. Later, impact of various factors such as carbon sources, nitrogen sources, pH and temperature has been assessed, and presented in the perspective of increasing its production from microbial strains. Finally, purification techniques for lipase enzymes was summarized and recent literature pertaining to lipase purification from microbial stains are reviewed, and summarized.

1. Introduction

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) are class of hydrolytic enzymes which catalyze the hydrolysis of insoluble triacylglycerol to glycerol, acylglycerols, and free fatty acids (Andualema and Gessesse, 2012; Akil et al., 2016; Geoffry and Achur, 2017). Lipases are a subclass of esterases and have long chain triacyl-glycerols, which is very low soluble in water and the reaction is catalysed at lipid-water interface (Aulakh and Prakash, 2010; Kumar and Kanwar, 2012). Lipases are highly efficient in the catalysing reactions in both aqueous and non-aqueous media due to their high stability in extremes of temperature, pH and organic solvents (Tan et al., 2018). Lipases are known to contain a hydrophobic lid, which is necessary for its interfacial activity (Khan et al., 2017).

Lipases are classified into two different categories based on the specificity and sources. Schematic representation of lipase classification has been shown in Fig. 1. Lipases have been found in many species of plants, animals, insects and microorganisms (Sahu and Martin, 2011; Sarmah et al., 2018). Lipases are extremely different in character and are ubiquitous in plants, animals and microorganisms (Zheng, 2018). The lipases, especially microbial lipase have gained much attention industrially than those derived from plants and animals due to their desirable characteristics and functional ability at extreme conditions, stability in organic solvent, chemo-selectivity, enantio-selectivity and they do not

require any co-factor (Thapa et al., 2019; Ramyasree and Dutta, 2013).

The high versatility of lipases, recognized as the most important group of biocatalysts in biotechnology field, which allows their applications in different industries like food, detergent, pharmaceutical, leather, textile, cosmetic, bio diesel production and paper industries (Kaur et al., 2016; Mouad et al., 2016; Avhad and Marchetti, 2019). The ability of lipases to perform very specific chemical and biological transformation has make them increasingly popular in those industries (De Abreu et al., 2014; Joseph et al., 2011; Maiangwa et al., 2015). Current review is investigated on microbial lipases, exploring the main methodologies used in screening and production process, enumerating influences of physical and chemical factors as well as purification techniques for these microbial lipases was discussed.

2. Microbial production of lipases

Among various lipases, microbial lipases have gained superior industrial attention due to their selectivity, stability, and broad substrate specificity (Treichel et al., 2010; Kumar et al., 2016). Many microorganisms are known as potential producers of lipases, including bacteria, fungi and yeast.

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2.1. Bacterial lipases

Many different Gram-positive and Gram-negative bacterial strains produce lipase enzyme. Some of the most commercially important lipase producing bacteria are recognized as belonging to the genera of *Bacillus*, which includes *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus alcalophilus*, *Bacillus coagulans*, *Bacillus stearothermophilus* and also some other bacterial strains such as *pseudomonas* sp., *Burkholderia* sp. and *Staphylococcus* sp. are also reported as better bacterial lipase producer (Suci et al., 2018; Sangeetha et al., 2010; Mabizela-Mokoena et al., 2017; Gowthami et al., 2015; Dror et al., 2015; Daoud et al., 2013). Lipase-producing bacterial strains has been found in different habitats such as oil industrial wastes, vegetable oil processing factories, dairy plants, paper industries and soil contaminated with oils. Tripathi et al. (2014) isolated eight different lipase producing bacteria from the pulp and paper mill industries. Recently, Bharathi et al. (2018) isolated five different lipase producing bacterial strains from petrol spilled soil. A review of the most recent potential bacterial strains for lipase production and their isolation area was reported in Table 1. According to the previous literature reports, oil contaminated sites are preferable for isolation of lipase producing bacterial strains. Furthermore, being nontoxic and eco-friendly, lipase are considered more suitable as compared to other chemical or synthetic catalysts. Therefore, widely used in food, dairy, flavour, detergent, pharmaceuticals, biofuels and cosmetics industries (Javed et al., 2018).

2.2. Fungal lipases

Fungal strains are known to be potential lipase producers with notable unique catalytic properties which are very important to various commercial applications (Pandey et al., 2016). Most of the commercially and industrially important lipase-producing fungi belong to the genera of *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp. (Riyadi et al., 2017; Oliveira et al., 2016; Pandey et al., 2016; Maldonado et al., 2016; Ülker and Karaoğlu, 2012). Lipase production by fungus varies according to the strain and composition of the growth medium such as carbon and nitrogen sources (Pandey et al., 2016).

Among microbial sources, filamentous fungus are good lipase producers and the extraction, purification, and processing steps are relatively simple. Roy et al. (2018) isolated a strain of *Aspergillus aculeatus*

from dairy waste contaminated soil and they obtained an expressive lipase producing activity of 9.51 U/ml.

The industrial demand for new sources of lipases with different catalytic character stimulates the isolation and selection of new fungal strains. Various lipase producing fungal strains are isolated by Colen et al. (2006) from Brazilian savanna soil by using enrichment culture techniques. A general view of the lipases from fungal sources and their isolation area are given in Table 1.

2.3. Yeast lipases

Lipase produced from yeast has unique applications in chemical, pharmaceutical and biodiesel producing industries (Singh and Mukhopadhyay, 2012). Recent literature survey shows, *Candida utilis*, *Candida rugosa*, *Rhodotorula* sp., *Yarrowia* sp. and *Pichia* sp. are the best and primary lipase producers (Moftah et al., 2012; Su et al., 2016; Divya and Padma, 2015; Lan et al., 2016; Fang et al., 2014; Resina et al., 2009). As per literature reports, *Candida* sp. is the most potential lipase producer from yeasts category. Biochemical, structural, and catalytic properties of *Candida* sp. lipase have been widely documented. He and Ten, (2006) investigated the production of lipase enzyme by *Candida* sp. and obtained an activity of 9.600 U/mL. Rajendran et al. (2008) documented the optimum lipase activity of 3.8 U/mL produced by *C. rugosa*. There are several publication reporting the production of lipase by yeasts and their isolation source are shown in Table 1.

3. Screening methods of lipase producer

Different strategies for lipase enzyme screening have been proposed and used for identifying microorganisms producing lipases which involve culturing of microbial strains on solid agar medium or in liquid media containing different substrates. Screening of lipase producing ability can be broadly classified in two different type, there are direct and indirect screening methods.

3.1. Direct method (qualitative-on agar medium)

Solid agar media with added substrates or indicator dyes can be used for screening of lipolytic microorganisms. It is a useful rapid method of screening to evaluate individual microorganisms for their lipase enzyme

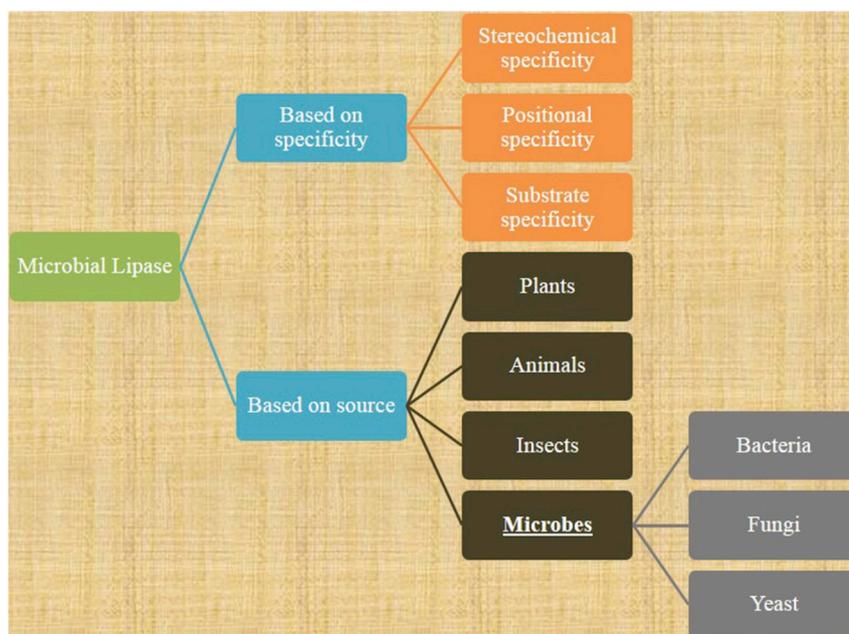


Fig. 1. Classification of microbial lipases based on sources.

Table 1
Microbial strains cited in the recent literature as potential lipase producer.

Microorganisms	Main source	Place of isolation/obtained	References
<i>Aeromonas caviae</i>	Bacteria	Milk processing industry	Velu et al. (2012)
<i>Acinetobacter</i> sp.	Bacteria	Oil rich soil	Ahmed et al. (2010)
<i>Bacillus sphaericus</i>	Bacteria	MTCC 7542	Tamilarasan and Kumar (2012)
<i>Bacillus</i> sp.	Bacteria	Oil contaminated soil	Sharma et al. (2014)
<i>Bacillus subtilis</i>	Bacteria	PTCC 1720	Esmaeili et al. (2015)
<i>Enterococcus durans</i>	Bacteria	Fish processing waste	Ramakrishnan et al. (2012)
<i>Staphylococcus warneri</i>	Bacteria	Oil-contaminated soil	Ye and Desai (2015).
<i>Thalassospira permensis</i>	Bacteria	Sea water	Kai and Peisheng (2016)
<i>Xanthomonas oryzae</i>	Bacteria	Soil sample	Mo et al. (2016)
<i>Yersinia enterocolitica</i>	Bacteria	Refrigerators of a meat factory	Ji et al. (2015)
<i>Aspergillus niger</i>	Fungi	NCIM	Hosseinpour et al. (2012)
<i>Meterhizium anisopliae</i>	Fungi	Dairy effluent	Colla et al. (2016)
<i>Penicillium chrysogenum</i>	Fungi	Agro-industrial residues	Rajeswari et al. (2011)
<i>Penicillium</i> sp.	Fungi	Soybean meal and cheese	Rigo et al. (2010)
<i>Rhizopus</i> sp.	Fungi	Oil contaminated soil	Thota et al. (2012)
<i>Rhizopus oryzae</i>	Fungi	Waste cooking oily soil	Zhou et al. (2012)
<i>Candida</i> sp.	Yeast	ATCC 10231	Lan et al. (2011)
<i>Cryptococcus</i> sp.	Yeast	MTCC 5455	Thirunavukarasu et al. (2016)
<i>Rhodotorula mucilaginosa</i>	Yeast	MTCC	Pohanka (2019)
<i>Saccharomyces cerevisiae</i>	Yeast	Olive oil site	Ciafardini et al. (2013)
<i>Trichosporon asahii</i>	Yeast	Petroleum sludge soil	Kumar and Gupta (2008)
<i>Yarrowia lipolytica</i>	Yeast	ATCC 20460	Lopes et al. (2008)
<i>Yamadazyma terventina</i>	Yeast	Olive oils site	Ciafardini et al. (2013)

Note: MTCC- Microbial type culture collection, PTCC= Persian type culture collection, NCIM- National Collection of Industrial Microorganisms, ATCC – American type culture collection.

production (Mateos-Díaz et al., 2012). Based on the use of substrates and indicator dye, direct plate assay further sub classified into two different types.

3.1.1. Gel diffusion assays using various lipid substrates

These methods are used for screening lipolytic microbial strains based on the ability of break down the lipids incorporated in to solid media to form a clear zone of lipolysis (Lee et al., 2015). Based on the substrates utilised, various agar plate assays has been established inducing substrates such as Olive oil, Tributyrin, Tween 20 and Tween 80 are greatly preferred by many researchers (Bharathi et al., 2018; Kumar et al., 2012a,b; Lanka and abd Trinkle, 2017). Among those substrates assays, Tributyrin agar plate assay has been widely used for detection of lipase production due to their easy preparation and time consuming (Bharathi et al., 2018). Smitha et al. (2014) screened various marine fungi cultures (181) for their ability to secrete hydrolytic enzymes using nutrient agar supplemented with Tributyrin as substrate and found that large number of isolated fungus to be having lipolytic activity.

3.1.2. Gel diffusion assays using various indicator dyes

These methods involve use of various indicator dyes with various

substrates to screen the lipase producing microbial strains. The dyes like phenol red, Rhodamine B, victoria blue, night blue can be used as indicator dye to screen. Rhodamine B added olive oil agar plate is a very common, sensitive and effective assay to identify active lipases. The basic principle and mechanism in this method involves interaction of hydrolyzed substrates with Rhodamine B (fluorescent dye) resulting in the formation of orange fluorescent halos around microbial colonies which can be visible upon UV irradiation (Dhiman and Chapadgaonkar, 2013; Kumar et al., 2012a,b; Niyonzima and More, 2013). Abrunhosa et al. (2013) reported that the combined Rhodamine B with olive oil to determine lipase production by *Aspergillus ibericus*. Screening of lipolytic microbes using indicator dye such as phenol red is also in wide practice.

3.2. Indirect method (quantitative-liquid medium)

Lipase producing ability of a microorganism can be determined using various quantitative methods such as titrimetric method, spectrophotometric method, chromatographic methods and molecular screening methods.

Titrimetric methods were routinely subjected to the estimation of lipase activity for more than half a century. Dayanandan et al. (2013) and Rigo et al. (2012) used alkali titration method to screen potential lipases producing *Penicillium crustosum* and *Aspergillus carneus* respectively. Recently, spectroscopic method using olive oil and p-NPP (p-nitrophenyl palmitate) as a substrate for quantitative screening potential lipase procedure has gained much attention due to their simple procedures (Velu et al., 2012; Romero et al., 2012). Rajan et al. (2011) quantified the liberated free fatty acids produced by the action of alkaline lipase from *Aspergillus fumigates* MTCC 9657 using HPTLC. Screening of various microbial strains using different qualitative and quantitative assay was shown in Table 2.

4. Production of microbial lipase

The microbial lipase are mostly extracellular and secreted into the lipase production medium. Submerged fermentation and solid state

Table 2
Screening methods of various lipase producing microorganisms.

Microorganisms	Screening method	Substrate	Reference
<i>Streptomyces violascens</i>	Spectrophotometer	p-NPP	Boran et al. (2019)
<i>Aspergillus tamari</i>	Titrimetric method	Olive oil	Das et al. (2016)
<i>Burkholderia pyrocinia</i>	Spectrophotometer	p-NPP	Li et al. (2014)
<i>Pseudomonas</i> sp.	Spectrophotometer	p-NPP	Priji et al. (2017)
<i>Rhizopus</i> sp.	Spectrophotometer	p-NPP	Riyadi et al. (2017)
<i>Bacillus</i> sp.	Titrimetric method	Olive oil	Saraswat et al. (2018)
<i>Bacillus sphaericus</i>	Spectrophotometer	p-NPP	Tamilarasan and Kumar (2012)
<i>Streptomyces</i> sp.	Titrimetric method	Olive oil	Kumar et al. (2016)
<i>Aeromonas caviae</i>	Spectrophotometer	p-NPP	Velu et al. (2012)
<i>Candida guilliermondii</i>	Spectrophotometer	p-NPP	Oliveira et al. (2016)
<i>Penicillium chrysogenum</i>	Gel diffusion - Indicator Dye	Rhodamine B	Rajeswari et al. (2011)
<i>Aspergillus sydawii</i>	Gel diffusion- lipid substrate	Tributyrin	Bindiya and Ramana (2014)
<i>Trichoderma reesei</i>	Gel diffusion- lipid substrate	Tributyrin	Rajesh et al. (2010)
<i>Aspergillus</i> sp.	Gel diffusion- lipid substrates	Palm oil and Tween-80	Nwuche and Ogbonna (2011)
<i>Aspergillus fumigates</i>	Chromatography (HPTLC)	–	Rajan et al. (2011)
<i>Pseudozyma Antarctica</i>	Molecular identification	–	Liu et al. (2010)

Note: p-NPP (para-Nitrophenylphosphate).

fermentation systems are widely used techniques to produce lipase enzyme from microorganisms.

4.1. Submerged fermentation system

Submerged fermentation system also called as liquid fermentation system. Submerged fermentation is a technique of cultivation of microorganisms in liquid broth medium which breaks down the supplied nutrients to compound (Costa et al., 2017). Further, the recovery and purification of lipases produced by SmF is relatively simple. SmF system mostly used for the production of lipase from bacterial strains. In commonly, oil carbon source such as vegetable oil, olive oil, coconut oil is common influencing factors in SmF system due to their sticky nature, even though various oils as a carbon source have been utilised to produce lipase enzyme from microbial strains especially olive oil, palm oil, sunflower oil, and almond oil has enhanced higher yield produced (Coradi et al., 2013; Colla et al., 2016; Salihu et al., 2012; Thakur, 2012).

4.2. Solid state fermentation system

Solid state fermentation system also called as solid substrate fermentation system and it is used several industries like food and pharmaceutical industries to produce compounds from microorganisms on solid substrate media (Mussatto et al., 2012; Barrios-González, 2012). Mostly, SSF used to produce fungal, mycelial and yeast lipases by using various solid substrate. For example, Sethi et al. (2016) used mustard oil as the substrate for lipase production by *Aspergillus sp.* in both SSF and liquid fermentation and the best result was observed in SSF. The advantages of producing lipase enzyme by SSF have been highlighted alongside the reduced production costs. Solid state fermentation (SSF) is an economical alternative for large scale production of enzymes that are produced by fungi. Therefore, production of lipases by solid state fermentation is a good and preferred option than submerged fermentation (Colla et al., 2015).

4.3. Factors influencing microbial lipase production

There are several factors strongly influence the production of lipase enzyme. The presence of an inducer particularly carbon sources as well as nitrogen sources and temperature, are the most considered factors.

4.3.1. Carbon source

In common, microbial lipase are produced upon induction of lipase producing genes. Carbon sources, particularly olive oil has a great role in induction of lipase enzyme in all type of microbial lipases. However, use of oil carbon sources (olive oil, palm oil, and other vegetable oils) for the induction of lipases has influence the recovery. A good yield of lipase production was observed by *Aspergillus terreus* when mustard seed oil used as a carbon source (Sethi et al., 2013). Combination of olive oil cake-sugar cane bagasse increases the lipase enzyme production in fungal strains. Enhancement of lipase production has been reported in bacterial strains when using olive oil compared to other (Zarevúcka, 2012). In contrast, Utilisation of Tween 80 has also aided in obtaining a better recovery of lipase produced by *Acinetobacter sp.* (Li et al., 2001). Along with vegetable oils are also widely used for better yield of higher lipases due to their cheap and readily available (Messias et al., 2009).

4.3.2. Nitrogen source

Nitrogen source is essential for microbial growth and enhancement of microbial lipase production. There are various organic and inorganic nitrogen sources such as ammonium salts, yeast extract, sodium nitrate, urea, peptone, tryptone, peptone has been used for the production of higher lipases from all form of microorganisms (Oliveira et al., 2016; Bose and Keharia, 2013; Priyanka et al., 2019; Das et al., 2016). Addition of urea to the culture medium for lipase production made the

culturing of *Rhizopus sp.* to have higher lipolytic activity (Rodriguez et al., 2006). Similarly, peptone combination with other nitrogen extract has been used for lipase production by *Aspergillus sp.* (Colonia et al., 2019).

4.3.3. Effect of temperature on lipase production

Temperature also play an important role in the production of microbial lipases. Optimum temperature plays a vital role for the secretion of enzyme in shake flask method. Higher biomass concentration of lipase was observed at temperature 37 °C (Bharathi et al., 2018). Researchers reported that the slight increase in temperature up to 38 °C enhances the lipase production (de Souza et al., 2019; Yuan et al., 2016). Lower temperature decrease the lipase enzyme production and also higher temperature influence the enzyme activity.

4.3.4. Effect of pH on lipase production

Generally, bacterial lipases have alkaline pH optima or neutral pH. Researchers reported that the alkaline and neutral pH conditions enhances the lipase productions in bacterial and yeast cells (Ramakrishnan et al., 2016; Bharathi et al., 2018). A good yield of lipase production was observed by *Rhodotorula glutinis* HL25 when pH of the production medium was maintained at nearly neutral pH (Taskin et al., 2016). In contrast, acidic pH enhances the production of fungal lipases. Turati et al. (2019) observed enhanced lipase production and activity while maintain pH of the reaction medium at 4.

5. Purification of lipase enzyme

Purification of microbial lipases involves various steps depending on the intracellular or extracellular nature of the enzyme. In either case, produced proteins are further salted out by precipitation techniques using ammonium sulphate (Yang et al., 2016). Further the precipitated protein is subjected to dialysis and chromatography techniques. The choice of chromatography techniques differ based on the source of microorganisms and size of the proteins, mostly column chromatography technique used to purify lipases. Finally the crude fractions thus obtained are analysed for the presence of enzyme by several assay protocols, and applied further separation techniques like ion exchange, DEAE-sepharose and gel filtration chromatography (Unni et al., 2016; Zhou et al., 2012). A recent separation techniques used to purify microbial lipase has been shown in Table 3. A lipase from *Aspergillus niger* F044 was purified by precipitation with ammonium sulphate, DEAE-Sephadex FF (ion exchange), and Sephadex G-75 (gel filtration). A yield of 33% was obtained, while the purification factor was 73 (Zheng-Yu et al., 2007).

The microbial lipases size vary from every microorganisms to others. In case of bacterial lipase produced from *Pseudomonas aeruginosa* expressed an average molecular weight of 29 kDa (Unni et al., 2016). Whereas the fungal lipase was found to be have average weight of 27 kDa (Zhou et al., 2012), Lipase also exists as a monomer with average weight of 46 kDa in yeast as reported by Kumar et al. (2014).

6. Conclusion

Analysis of current literature shows microbial lipases are one of the most produced enzymes. Globally, many researches are conducting their studies on isolation, screening and optimizing the critical growth parameters for microbial strains to gain maximum yield of lipases. Qualitative screening has been achieved by using agar plate assay method with various substrates such as olive oil, Tributyrin and Tween 20/80. Further, quantitative screening has been achieved by culturing microorganisms in a liquid medium and subsequently identifying the lipase activity using various colorimetric and spectrophotometric methods. This type of screening methods, especially colorimetric methods helped to identify microbial strains which continuously produce lipases with higher yield. Production of microbial lipases can be carried out using

Table 3
Purification strategies recently used for various studies of microbial lipases.

Microorganisms	Purification techniques	Molecular weight (kDa)	References
<i>Burkholderia ubonensis</i>	Ammonium sulphate precipitation, anion exchange and sephadex 75 gel filtration chromatography	33	Yang et al. (2016)
<i>Pseudomonas aeruginosa</i>	Ammonium sulphate precipitation and sephadex G-100 gel filtration	29	Unni et al. (2016)
<i>Idiomarina</i> sp.	Ammonium sulphate precipitation, DEAE-sepharose and sephacryl S-200	67	Li et al. (2014)
<i>Bacillus</i> sp.	Ammonium sulphate precipitation and ion-exchange chromatography	24	Sivaramakrishnan and Incharoensakdi (2016)
<i>Chromohalobacter</i> sp.	Ammonium sulphate precipitation, sephacryl S-100	44	Xin and Hui-Ying (2012)
<i>Penicillium</i> sp.	Dialysis, Octyl Sepharose FF column	65.4	Daniela Flávia and M. Turati et al. (2019)
<i>Aspergillus oryzae</i>	Ammonium sulphate precipitation, DEAE-sepharose and sephadex G-100 gel filtration	27	Zhou et al. (2012)

Note: kDa denotes kilo Dalton.

both solid state and submerged fermentation system using various carbon and nitrogen source. Most of the literature reports supported that the solid state fermentation systems are better methods for microbial lipase production. The nutritional factors influencing microbial lipase production have been explored and have shown to be integral in enhancing lipase yield. Finally purification of lipases using different precipitation and chromatography techniques has been discussed and summarized. The microbial lipases have many applications and benefits in the food and agro-industries. The key advantage of choosing lipase as a biocatalyst in a number of organic transformations and also used for biodiesel production process.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101368>.

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