Screening of Lipase Activities with Cultures from the Agricultural Research Service Culture Collection

C.T. Hou* and **T.M. Johnston**

Oil Chemical Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

A simple, sensitive agar plate method was used to screen lipase activity from 1229 selected cultures, including 508 bacteria, 479 yeasts, 230 actinomycetes and 12 fungi, covering many genera and species. About 25% of the cultures tested are lipase-positive. These lipase-positive strains were further classified into three categories according to their enzyme activity: good, moderate and weak lipase producers for those having orange fluorescent halo zones greater than 10 mm, 7.5 mm or 5 mm diameters, respectively. The good lipase producers have the potential to be developed for industrial enzymes. The positional, fatty acid or enantiospecificity of each individual lipase requires further investigation. The data presented here are important for the discovery of new lipases.

KEY WORDS: Actinomycetes, agar-plate screening method, bacteria, lipases, yeasts.

Early research on microbial lipases focused on prevention of food spoilage, but recent work attempts to provide less expensive stable industrial lipases. Currently, most lipases are used in the medical, food and detergent industries. Although pancreatic lipase and oral lipase (from bovine) can be used for these purposes, less expensive microbial lipase is currently the main source for these industries. Lipases catalyze the hydrolysis of triglycerides, or acyl and aryl esters. Although most work is with aqueous systems, lipases also are capable of catalyzing organic reactions in nonaqueous media (1). Lipases hydrolyze and synthesize triglycerides with positional and fatty acid specificities. Okumura *et al.* (2) reported that lipases from *Aspergillus niger* and *Rhizopus delemar* hydrolyzed or formed ester bonds only at positions 1 and 3 of glycerol. In contrast, lipases from *Geotrichum candidum* and *Penicillium cyclopium* hydrolyzed and formed ester bonds at all three positions. Bloomer *et al.* (3) studied twelve commercially available lipases for their ability to interesterify triglycerides, thus changing their fatty acid composition and physical properties.

Most lipases consist of isozymes. Baillargeon (4) fractionated commercial lipase from *G. candidum* into five isozymes. Four isozymes are specific for oleate *vs.* stearate esters, and one isozyme is specific for oleate *vs.* palmitate. Yamaguchi and Mase (5) reported mono- and diacylglycerol lipase from *Penicillium camembertii* U-150 that was completely inert to triglycerides. Although the natural substrates of lipases are acylglycerols, they also can catalyze the hydrolysis of a wide range of artificial water-insoluble esters with a high degree of enantiospecificity.

In recent years, interest in the use of enzymes as hydrolytic or synthetic chiral catalysts has risen rapidly. The extracellular microbial lipases are particularly suited for this application. Because of their availability, the more than one dozen commercially available lipases have been studied repeatedly by researchers in industry and in academia. For example, Sih *et al.* (6) studied several commercial lipases for the enantiospecific hydrolysis of 2-arylpropionic acid methyl esters, an important class of nonsteroidal anti-inflammatory drugs, and aroyl-thio-2S-methyl-propionic acid methyl esters, key chiral intermediates for the synthesis of the antihypertensive agent captopril. Barton *et al.* (7) studied several commercial lipases for enzymatic resolution of phenoxy compounds such as (R,S)-methyl-2-(4-hydroxyphenoxy) propionic acid methyl esters, a valuable intermediate in the production of certain herbicides. However, under most conditions the enantioselectivity has been too low to permit economical production of the desired enantiomer.

Lipases can also function as esterases. For example, Tsujisaka *et al.* (8) reported that *A. niger* lipase synthesizes glycerides of DL-maleic acid, succinic acid and aromatic acids, such as benzoic acid and phenylacetic acid. In contrast, lipases from *C. candidum* and *P. cyclopium* synthesize only glycerides of long-chain fatty acids. Lipases from *A. niger* and *R. delemar* synthesize oleyl alcohol esters of various fatty acids and some dibasic acids, whereas lipases from *G. candidum* and *P. cyclopium* synthesize oleyl esters of medium- or long-chain fatty acids.

Alcohol specificities of lipases also have been noted. Okumura *et al.* (2) reported that lipases from *A. niger*, *R. delemar*, *G. candidum* and *P. cyclopium* synthesize various esters from oleic acid and from many primary alcohols. However, only *G. candidum* lipase synthesized oleic acid esters of secondary alcohols. These four lipases did not synthesize esters of tertiary alcohols, phenols or sugars. A relatively high alcohol concentration is required to synthesize esters of ethylene glycol, propylene glycol or trimethylene glycol. *Aspergillus niger* lipase is most suitable for synthesis of terpene alcohol esters of short-chain fatty acids. Synthesis of fatty hydroxamic acid from fatty acid and hydroxylamine by a *Mucor* lipase has also been reported (9).

The need for novel lipases/esterases is obvious, and industry continues to look for high activity and less expensive microbial sources. New lipases from microbial sources have been reported sporadically (10–12). But to our knowledge, no effort has been made or is being planned for conducting a large-scale, systematic screening for new lipases/esterases. This is due, in part, to the fact that such screening efforts may lead to uncertain results and that large numbers of cultures are not readily available to one scientist. At NCAUR (Peoria, IL) with the support of Biotechnology Research & Development Corporation (Peoria, IL), we had the opportunity to conduct a large-scale screening for lipase/esterase activity with cultures in the ARS Culture Collection (Peoria, IL).

Known lipase-activity screening methods, such as titrimetric and colorimetric (13,14), are time-consuming and cannot be used for a large-volume screening operation. Screening of lipase activity on agar plates also has been reported with Tween 80 in combination with Nile-blue (15) or neat's foot oil with Cu^{2+} salts (16). However, those substrates are not suitable to detect true lipases because they are hydrolyzed by esterases. A fluorescent dye rhodamine B was used

*To whom correspondence should be addressed at NCAUR, 1815 N. University St., Peoria, IL 61604.

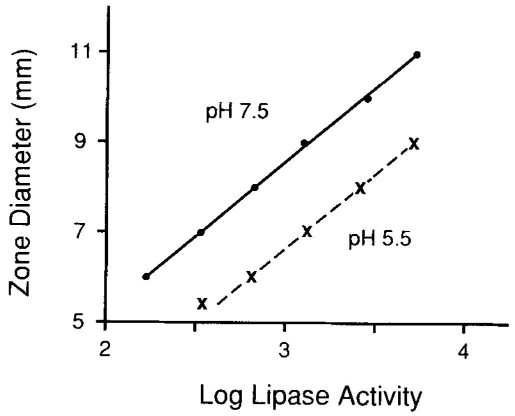

FIG. 1. Relationship between log lipase activity and the diameter of orange fluorescent halos. Sigma lipase type II (pancreatic) in 3 μL water was used. The lipase activities were 2.65, 1.32, 0.66, 0.33, 0.16 and 0.08 units, respectively.

to visualize fungal lipase on an ultrathin-layer isoelectro focusing gel (17). Subsequently, this technique was adapted into a specific and sensitive plate assay for bacterial lipase activity (18).

We have used a modified rhodamine B agar plate method to screen 1229 selected cultures covering many genera and species of bacteria, yeasts, actinomycetes and fungi. About 25% of the cultures screened were lipase-positive. These cultures were further classified into three categories according to their lipase activities. This paper describes a screening method and the results of the screening operation.

MATERIALS AND METHODS

Microorganisms. All microbial cultures were obtained from the ARS Culture Collection. Bacteria were grown on Triton glucose yeast extract (TGY) medium which contained (per liter): 5 g tryptone, 5 g yeast extract, 1 g dextrose, 1 g K_2HPO_4, pH 7.5 at 30°C. Yeasts and actinomycetes were grown on potato dextrose agar (PDA) medium which contained (per liter): 26 g PDA (Difco Lab, Detroit, MI), pH 5.5 at 25°C. For preparing agar plates, 10 g agar was added into the respective medium.

Chemicals. Commercial lipases (triacylglycerol lipases, EC 3.1.1.3.), rhodamine B, soybean oil, corn oil and olive oil were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent-grade and were used without further purification.

Lipase activity screen. The media mentioned above were autoclaved, and then cooled to about 60°C. Then, 30 mL of filter-sterilized triacylglycerol and 2 mL of 0.1% filter-sterilized rhodamine B in H_2O were added with vigorous shaking. After the medium was allowed to stand for 10 min at 60°C to reduce foaming, 20 mL was poured into each plate. Cultures were inoculated as a small spot on the screening agar plate and incubated at 30°C for bacteria and 25°C for yeasts and actinomycetes. Lipase activity was identified on the plate as an orange fluorescent halo zone after 48 h of incubation. As a control, various quantities of a commercial pancreatic lipase were assayed with this screening plate. Their orange fluorescent halo zone sizes correlated well with their lipase activities (Fig. 1).

RESULTS

Screening results on yeasts and bacteria plates can be seen in Figure 2, A and B, respectively. It is easy to identify the orange fluorescent halos and to pick up strains with good lipase activity. One-thousand two-hundred and twenty-nine selected cultures, including 508 bacteria, 479 yeasts, 230 actinomycetes and 12 fungi, covering many genera and species, were screened. Table 1 shows the

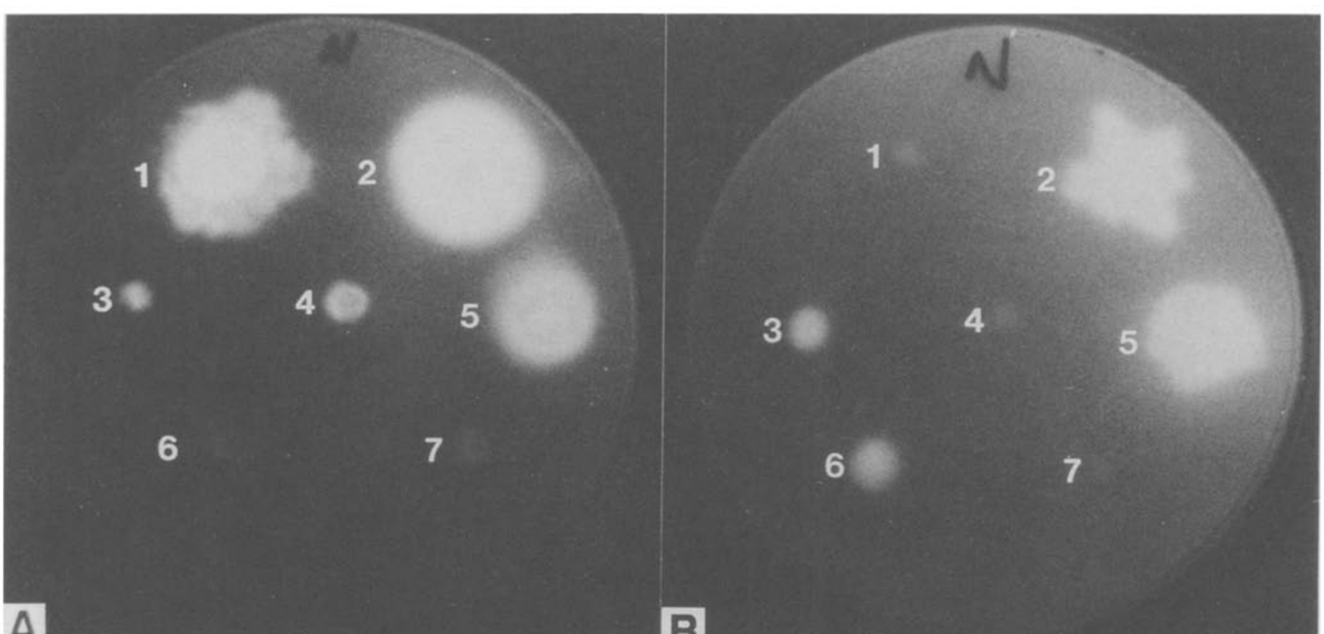

FIG. 2. Rhodamine B agar plates screen for lipase activity. A, pH 5.5 for yeasts: 1, *Yarrowia lipolytica* Northern Regional Research Lab (NRRL) Y-1095; 2, *Geotricum candidum* NRRL Y-552; 3, *Candida magnoliae* NRRL Y-2024; 4, *C. magnoliae* Y-4226; 5, *C. azyma* NRRL Y-17067; 6, *C. krusei* NRRL Y-17013; and 7, *C. lambica* NRRL Y-100. B, pH 7.5 for bacteria: 1, *Pseudomonas acidovorans* NRRL B-783; 2, *Ps. fluorescens* NRRL B-1800; 3, *Ps. fluorescens* NRRL B-1796; 4, *Ps. acidovorans* NRRL B-819; 5, *Ps. myxogenes* NRRL B-2086; 6, *Ps fluorescens* NRRL B-1882; and 7, *Ps. putida* NRRL B-2024.

TABLE 1

Microorganisms Screened for Lipase Activity

Microorganisms	Number of strains tested	Number of strains positive	Microorganisms	Number of strains tested	Number of strains positive
Yeasts:			*C. insectalens*	1	0
Candida acuta	1	0	*C. insectamans*	1	1
C. acudensis	1	1	*C. kefyr*	3	0
C. antarctica	1	1	*C. krissii*	2	1
C. apicola	3	1	*C. krusei*	2	0
C. apis	1	0	*C. lactiscondensi*	1	0
C. atmospherica	1	1	*C. lambica*	4	0
C. auringiensis	2	0	*C. lipolytica*	2	0
C. azyma	1	1	*C. lodderae*	1	0
C. bondarzwiae	1	0	*C. lusitaniae*	1	1
C. boidinii	1	0	*C. magnoliae*	6	6
C. boleticola	1	1	*C. maltosa*	1	1
C. bombi	1	1	*C. mannitofaciens*	1	0
C. bombicola	2	2	*C. marina*	1	0
C. buffonii	1	1	*C. maris*	1	0
C. buinensis	1	0	*C. maritima*	2	1
C. butyri	1	0	*C. melinii*	4	3
C. cacaoi	1	1	*C. membranaefaciens*	4	0
C. cantarelli	2	0	*C. mesenterica*	1	0
C. cariosilignicol	1	0	*C. methanosorbosa*	1	0
C. castellii	1	0	*C. methylica*	1	1
C. castrensis	1	0	*C. milleri*	1	0
C. chilensis	2	1	*C. mogii*	2	0
C. chiropterorum	1	0	*C. molischiana*	1	0
C. colliculosa	1	0	*C. montana*	1	0
C. conglobata	1	0	*C. mucilagina*	2	1
C. dendrica	1	1	*C. musae*	1	0
C. dendronema	1	1	*C. naeodendra*	1	1
C. deserticola	1	0	*C. navarrensis*	1	0
C. diddensiae	1	0	*C. nemodendra*	1	0
C. diddensii	5	0	*C. nitratophila*	1	0
C. diffluens	1	0	*C. nodaensis*	1	1
C. diversa	2	0	*C. norvegica*	1	1
C. edax	1	0	*C. oleophila*	3	0
C. entomaea	1	1	*C. oregonensis*	2	1
C. entomophila	1	1	*C. kpalmioleophila*	1	0
C. eremophila	1	0	*C. pampelonensis*	1	0
C. ernobii	2	1	*C. parapsilosis*	2	2
C. etchellsii	2	0	*C. philyla*	1	1
C. ethanolica	1	0	*C. pinus*	1	0
C. famata	4	0	*C. podzolica*	1	0
C. fennica	1	0	*C. pseudointermedia*	1	0
C. fermenticarens	1	0	*C. pseudolambica*	1	0
C. flavificans	1	0	*C. ptarmiganii*	1	0
C. fluviatilis	3	0	*C. pulcherrima*	1	0
C. fragariorum	1	0	*C. quercitrusa*	1	1
C. freyschussii	1	1	*C. quercuum*	1	1
C. fructus	1	0	*C. rhagii*	4	0
C. fusiformata	1	1	*C. rugopelliculosa*	1	0
C. geochares	1	1	*C. rugosa*	1	0
C. glabrata	2	0	*C. saitoana*	1	0
C. glucosophila	1	0	*C. sake*	12	0
C. gropengiesseri	1	0	*C. salmanticensis*	1	1
C. guilliermondii	4	0	*C. santamariae*	1	1
C. haemulonii	1	0	*C. savonica*	1	1
C. halophila	1	0	*C. schatavii*	1	0
C. hellenica	2	2	*C. shehatae*	2	0
C. homilentoma	1	1	*C. silvanorum*	1	1
C. humicola	3	2	*C. silvatica*	1	0
C. humilus	1	0	*C. silvicola*	2	2
C. hydrocarbofumarica	1	0	*C. silvicultrix*	1	1
C. hylophila	1	0	*C. solani*	5	0
C. incommunis	1	0	*C. sonorensis*	2	0
C. inconspicua	1	0	*C. sorbophila*	1	0
C. ingens	3	0	*C. spandovensis*	1	0
C. insitophila	1	0	*C. species*	7	3

(continued)

TABLE 1 (continued)

Microorganisms	Number of strains tested	Number of strains positive	Microorganisms	Number of strains tested	Number of strains positive
Yeasts:			*P. kluyveri*	1	0
C. steatolytica	7	0	*P. lynferdii*	1	1
C. stellata	3	0	*P. media*	2	0
C. subtropica	1	0	*P. membranaefaciens*	2	1
C. succiphila	2	0	*P. methanolica*	1	0
C. tanzawaensis	1	1	*P. mexicana*	2	2
C. tenuis	2	0	*P. meyerae*	1	1
C. torresii	1	0	*P. minuta*	3	1
C. tropicalis	2	0	*P. mississippiensis*	2	2
C. tsuchiyae	1	0	*P. muscicola*	2	2
C. tsukubaensis	1	0	*P. naganishii*	1	0
C. utilis	2	0	*P. nakasei*	1	0
C. vartiovarrai	1	0	*P. nakazawae*	2	1
C. versatilis	1	0	*P. norvegensis*	1	0
C. vini	1	0	*P. ohneri*	2	0
C. wickerhamii	3	0	*P. onychis*	2	0
Geotrichum candidum	1	1	*P. opuntiae*	4	0
Issatchenkia orientalis	2	0	*P. pastoris*	2	0
I. scutulata	3	0	*P. petersonii*	1	1
I. terricola	1	0	*P. pinus*	2	0
Moniliella suaveolens	1	0	*P. populi*	1	0
Pachysolen tannophilus	2	0	*P. quercuum*	1	0
Pachytichospora transvaalensis	1	0	*P. rabaulensis*	1	1
Pichia abadieae	1	1	*P. rhodanensis*	2	0
P. acaciae	2	0	*P. saitoi*	3	0
P. alni	2	1	*P. salicornia*	1	0
P. amenthionina	2	0	*P. salictaria*	2	0
P. americana	2	2	*P. sargentensis*	1	0
P. amylophila	1	0	*P. scolyti*	1	0
P. angophorae	2	0	*P. segobiensis*	2	0
P. angusta	4	0	*P. silvicola*	2	2
P. anomala	2	2	*P. sosrbitophila*	1	0
P. antillensis	2	0	*P. spartinae*	2	0
P. besseyi	1	0	*P. stipitis*	2	0
P. bimundalis	2	2	*P. strasburgensis*	2	0
P. bispora	2	2	*P. subpelliculosa*	2	0
P. bovis	2	0	*P. sydowiorum*	1	1
P. burtonii	2	0	*P. toletana*	2	2
P. cactophila	2	0	*P. trehalophila*	2	0
P. canadensis	3	2	*P. veronae*	1	1
P. capsulata	2	0	*P. wickerhamii*	2	0
P. carsonii	2	0	*P. xylosa*	5	5
P. castillae	1	0	*P. zaruensis*	2	0
P. cellobiosa	2	0	*Saccharomyces albasitensis*	1	0
P. chambardii	1	0	*S. astigiensis*	1	0
P. ciferrii	1	0	*S. cervisiae*	2	0
P. delftensis	1	0	*S. placentae*	1	0
P. deserticola	2	0	*Saccharomycopsis capsularsis*	2	0
P. dispora	2	0	*S. crataegensis*	2	2
P. dryadoides	2	1	*S. fibuligera*	3	1
P. etchellsii	2	0	*S. malanga*	1	0
P. euphorbiophila	2	2	*S. placentae*	1	0
P. fabianii	2	2	*S. synnaedendra*	1	0
P. farinosa	2	0	*Trichosporon species*	2	2
P. fermentans	1	0	*Williopsis beijerinckii*	2	0
P. finlandica	1	0	*W. dimenae*	2	0
P. fluxuum	2	0	*W. mrakii*	2	0
P. fluxuum	2	0	*W. pratensis*	1	0
P. glucozyma	2	1	*W. saturnus*	3	0
P. guilliermondii	1	0	*W. species*	1	0
P. hampshirensis	1	1	*Wickerhamiella domercqiae*	2	1
P. haplophila	2	0	*Wingea robertsii*	2	0
P. heedii	2	0	*Yarrowia lipolytica*	4	4
P. henricii	2	0	*Zygosaccharomyces bailii*	12	0
P. holstii	2	2	*Z. bisporus*	1	0
P. inositovora	1	1	*Z. cidri*	4	0
P. jadinii	1	0	*Z. fermentati*	6	1
P. japonica	2	0	*Z. florentinus*	2	0

(continued)

TABLE 1 (continued)

Microorganisms	Number of strains tested	Number of strains positive	Microorganisms	Number of strains tested	Number of strains positive
Yeasts:			*P. myxogenes*	7	6
Z. microellipsoides	5	0	*P. nonliquefaciens*	4	2
Z. mrakii	2	0	*P. oleovorans*	1	0
Z. rouxii	11	0	*P. oxalaticus*	3	0
Bacteria:			*P. pantotropha*	1	0
Acinetobacter calcoaceticus	1	0	*P. pavonacea*	4	1
A. species	1	0	*P. perolens*	2	1
Alcaligenes faecalis	1	1	*P. putida*	29	11
Alteromonas putrefaciens	11	1	*P. putrifaciens*	9	0
A. species	6	2	*P. reptilovora*	18	10
Arthrobacter citreus	2	0	*P. resinovorans*	1	1
A. globiformis	8	0	*P. ribicola*	2	0
A. luteus	1	0	*P. ribis*	3	0
A. simplex	3	0	*P. riboflavina*	3	1
A. species	3	0	*P. rubescens*	2	0
A. terregens	2	1	*P. saccharophila*	1	1
A. tumescens	1	0	*P. seminum*	1	1
A. viscosus	2	0	*P. septica*	4	4
Bacillus amyloliquefaciens	13	5	*P. species*	13	9
B. aneurinolyticus	1	0	*P. striafaciens*	1	1
B. brevis	11	0	*P. stutzeri*	2	1
B. cereus	18	0	*P. ssuis*	7	1
B. circulans	4	1	*P. syncyanae*	3	1
B. firmus	7	0	*P. taetrolens*	3	0
B. globiformis	1	0	*P. testosteroni*	1	0
B. indoloxidans	1	0	*P. tolaasii*	1	1
B. laterosporus	1	0	*P. viridilivida*	5	2
B. licheniformis	18	0	*P. viscosa*	3	2
B. macerans	3	0	Unknown species	30	1
B. megaterium	19	8	Actinomycetes:		
B. polymyxa	10	0	*Actinomyces* sp.	1	0
B. pulvifaciens	4	0	*Actinoplanes arizonaensis*	1	0
B. pumilus	14	6	*A. armeniaca*	1	0
B. species	3	0	*A. missouriensis*	1	0
B. stearothermophilus	3	0	*A.* sp.	3	0
B. subtilis	19	7	*A. violaceus*	1	0
Brevibacterium species	2	0	*A. yunnanensis*	1	0
Corynebacterium species	1	0	*Amycolata autotrophica*	1	0
Enterobacter aerogenes	1	0	*A. hydrocarbonoxydans*	2	0
E. oxytoca	1	0	*Amycolatopsis orientalis*	2	0
Flavobacterium aurantiacum	1	1	*A. saturnae*	1	0
Lactobacillus casei	1	0	*A.* sp.	3	0
Mycobacterium species	2	0	*Brevibacterium* sp.	2	0
Pseudomonas aceris	2	0	*Chainia antibiotica*	1	0
P. acidovorans	8	1	*C. aurea*	1	0
P. aeruginosa	38	33	*C. flava*	1	0
P. amylodermosa	1	0	*C. fumigata*	1	0
P. antimycetica	1	1	*C. kunmingensis*	1	0
P. aromatica	2	0	*C. nigra*	1	0
P. aureofaciens	5	0	*C. ochracea*	1	0
P. berberidis	1	1	*C. olivacea*	1	0
P. calcoacetica	1	0	*C. poonensis*	1	1
P. cepacia	1	0	*C. purpurogena*	2	1
P. chlororaphis	14	10	*C. rubra*	1	0
P. dacunhae	1	0	*C. voilens*	1	1
P. delafieldii	1	0	*Citermyces matritensis*	2	0
P. diminuta	1	0	*Paecilomyces lilacious*	4	3
P. echinoides	1	1	*Kitasatoa griseophaeus*	1	0
P. excibis	1	0	*Lodderomyces elongisporus*	2	0
P. fluorescens	39	24	*L. opuntiae*	2	0
P. fragi	5	4	*Micrococcus species*	12	0
P. indigoera	4	0	*Micromonospora aurantiaca*	1	0
P. maltophila	1	0	*M. brunnea*	1	0
P. marginata	4	1	*M. brunnescens*	1	0
P. mephitica	1	0	*M. capillata*	1	0
P. mexicana	1	1	*M. chalcea*	1	0
P. mildenbergii	4	0	*M. species*	19	0
P. mucidolens	2	1	*M. thermauberginospora*	1	0

(continued)

TABLE 1 (continued)

Microorganisms	Number of strains tested	Number of strains positive	Microorganisms	Number of strains tested	Number of strains positive
Actinomycetes:			*S. placentae*	1	0
M. yulongensis	1	0	*Saccharothrix aerocolonigenes*	1	0
Moniliella suaveolens	1	0	*Streptomyces aculentosporus*	1	1
Mycobactereium sp.	2	0	*S. afghaniensis*	1	1
Nocardia acidohilus	1	0	*S. agglomeratus*	2	0
N. amarae	3	0	*S. ahygroscopicus*	1	0
N. aurantia	2	2	*S. akiyoshiensis*	2	0
N. calcarea	1	0	*S. albogriseolus*	4	1
N. cellulans	1	0	*S. albus*	13	1
N. coeliaca	1	0	*S. argentolus*	1	0
N. convoluta	1	0	*S. aureofaciens*	9	0
N. farcinica	4	0	*S. aureus*	3	2
N. flavorosea	1	0	*S. bolili*	1	0
N. gibsonii	2	1	*S. bottropensis*	1	0
N. graminis	1	0	*S. cacaoi*	1	0
Norcardia nitrificans	1	0	*S. canescans*	1	0
N. pseudosporandigera	1	0	*S. caniferus*	1	0
N. species	2	0	*S. canus*	1	0
Pachysolen tannophilus	2	0	*S. capoamus*	1	1
Pachytichospsora transvaalensis	1	0	*S. carneus*	2	1
Pseudoamycolata halophobica	1	0	*S. falvovirens*	4	1
P. species	1	0	*S. gelaticus*	1	0
R. chlorophenolicus	1	0	*S. geysiriensis*	1	0
R. coprophilus	1	0	*S. griseus*	15	1
R. equi	2	0	*S. lavendulae*	16	0
R. erythropolis	3	0	*Torulaspora delbrueckii*	2	0
R. rhodnii	1	0	*T. globosa*	1	0
R. rhodochrous	5	1	*T. pretoriensis*	1	0
Rhodococccus rubropertinctus	1	0	Unknown species	21	2
R. spe.	1	0	Fungi:		
Saccharomyces albasitensis	1	0	*Aspergillus sydowi*	4	0
S. astigiensis	1	0	*Penicillium citrinum*	4	4
S. cerevisiae	2	0	*P. funiculosum*	4	4

species and number of strains screened and the number of strains that were lipase positive.

Among the 1229 cultures screened, 168 bacteria, 119 yeasts, 22 actinomycetes and 6 fungi were found to be lipase-positive. These cultures were further classified by their enzyme activities into good lipase producers (those cultures having an orange fluorescent halo zone diameter greater than 10 mm), moderate lipase producers (those cultures having halo zone diameters between 7.5 and 10.0 mm) and weak lipase producers (those cultures having halo zone size between 5 and 7.5 mm diameters) (Tables 2, 3 and 4). Classification of these lipases according to their positional, fatty acids or chiral specificities requires further studies. Because the substrates for lipase differ depending on areas of research or a company's need, further classification of these lipases will be left to the individual scientist. Nevertheless, the data presented here provide important information for the discovery of new lipases.

DISCUSSION

We described here a simple, specific and large-volume screening method for lipase activity. The binding of rhodamine B dye and fatty acids and mono- or diglycerides is highly sensitive and specific (18). The mechanism of this orange fluorescent compound formation is not known. However, Kouker and Jaeger (18) suggested that rhodamine B dimers complex with fatty acid and mono- or diglycerides. We have kept the agar content in the screening media low to increase the diffusibility of extracellular lipases in the agar plate. We tested four potential substrates-soybean oils, corn oil, olive oil and tributyrin—on our screening plate against several commercial lipases (type II porcine pancreatic, type VII *Candida cylindracea* and type XIII *Pseudomonas* sp.). All of these substrates were found suitable to be used for screening these types of lipases. We selected soybean oil as our screening substrate. About 25% of the screened cultures possess lipase activity. Fungi have a higher percentage for good lipase producers per number of cultures tested (25%) than do bacteria (13.7%), yeasts (2.6%) and actinomycetes (2.6%). Those good lipase producers are candidates for exploring their potential as industrial lipases. However, for a new lipase that has more specific activities such as positional, fatty acid and enantiospecificities, one should not overlook those strains that are moderate and weak lipase producers.

TABLE 2

Microorganisms That Produce Orange Fluorescent Zones Equal To and Larger Than 5 mm in Diameter

Microorganisms	Northern Regional Research Lab (NRRL) No.
Bacteria:	
Alcaligenes faecalis	B-1965
Altermonas species	B-956, B-973
Arthrobacter terregens	B-14092
Bacillus amyloliquefaciens	B-1466
B. circulans	B-383
B. megaterium	B-47, B-1367, B-1827
B. pumilus	B-208, B-3480, B-3481
B. subtilis	B-361, B-364
Pseudomonas acidovorans	B-979
P. aeruginosa	B-248, B-249, B-256, B-275, B-282, B-450, B-451
P. antimycetica	B-1683
P. beerberidis	B-831
P. chlororaphis	B-1097, B-1541, B-1869, B-18, B-2266
P. echinoides	B-3127
P. fluorescens	B-1105, B-1608, B-1897, B-1964, B-2547, B-2640, B-4290
P. mexicana	B-4222
P. nonliquefaciens	B-993
P. pavonacea	B-724
P. putida	B-13, B-254, B-931, B-1245, B-2023, B-2079, B-2174, B-2336
P. resinovorans	B-2649
P. seminum	B-2742
P. septica	B-1963, B-2082
P. species	B-1794, B-1878, B-1880 B-1881, B-1883
P. suis	B-927
P. syncynanea	B-1246
P. viscosa	B-2538
P. viridilivida	B-893
Yeasts:	
Candida ancudensis	Y-17327
C. antarctica	Y-7954
C. atmospherica	Y-5979
C. boleticola	Y-17080
C. bombi	Y-17081
C. bombicola	Y-5391, Y-17069
C. buffoni	Y-17082
C. chilensis	Y-17141
C. dendrica	Y-7775
C. entomaea	Y-7785
C. entomophila	Y-7783
C. ernobii	Y-12941
C. fusiformata	Y-17173
C. geochares	Y-17073
C. hellenica	Y-6591, Y-17319
C. homilentoma	Y-10941
C. humicola	Y-1266
C. insectamans	Y-7786
C. krissii	Y-1708
C. lusitaniae	Y-7940
C. magnolia	Y-680, Y-2024, Y-7621, Y-7622
C. maritima	Y-7899
C. mucilagina	Y-11823
C. naeodendra	Y-10942
C. nodaensis	Y-2484
C. norvegica	Y-6700
C. oregonensis	Y-5850
C. parapsilosis	Y-7629, Y-7659
C. philyla	Y-7776
C. salmanticensis	Y-17090
C. santamariae	Y-6656
C. savonica	Y-17077
C. silvicola	Y-6052

(continued)

TABLE 2 (continued)

Microorganisms	NRRL No.
Yeasts:	
C. silvicultrix	Y-7789
C. sp.	YB-599, Y-490
C. tanzawaensis	Y-17324
Pichia abadiae	Y-7499
P. alni	Y-11625
P. anomala	Y-366
P. bimundalis	YB-2805
P. bispora	Y-1482, Y-11610
P. dryadoides	Y-10990
P. euphorbiophila	Y-12742, Y-12743
P. fabianii	Y-1871, Y-1873
P. glucozyma	YB-2185
P. hampshirensis	YB-4128
P. inositovora	Y-12698
P. mexicana	Y-11818, Y-11819
P. meyerae	Y-12777
P. minuta	Y-10948
P. mississippiensis	YB-1294
P. nakazawae	Y-7904
P. silvicola	YB-3086
P. toletana	YB-4247, Y-11549
P. veronae	Y-7818
P. xylosa	YB-3884, YB-3887, Y-5987, Y-12929, Y-12939
Saccharomycopsis crataegensis	Y-5902
S. fibuligera	Y-12677
Trichosporon species	Y-1489
Wickerhamiella domercqiae	Y-6698
Actinomycetes:	
Unknown species	B-12699, B-7659
Chainia poonensis	B-2319
C. purpurogena	B-2952
C. violens	B-3483
Nocardia gibsonii	B-2592
Rhodococcus rhodochrous	B-16562
Streptomyces albus	B-2380
S. carneus	B-2001
S. flavovirens	B-2685
S. griseus	B-2027
Fungi	
Penicillium citrinum	6336
P. funiculosum	13095

TABLE 3

Microorganisms That Produce Orange Fluorescent Zones Equal To and Larger Than 7.5 mm in Diameter

Microorganisms	Northern Regional Research Lab (NRRL) No.
Bacteria:	
Alteromonas putrefaciens	B-951
Bacillus subtilis	B-542
Flavobacterium aurantiacum	B-184
Pseudomonas aeruginosa	B-7, B-12, B-23, B-27, B-79, B-211, B-247, B-250, B-265, B-452, B-534, B-3509, B-4014
P. chlororaphis	B-560, B-561, B-1632
P. fluorescens	B-804, B-1244, B-1603, B-1609, B-1612, B-1636, B-1796, B-1944, B-2322
P. putida	B-8, B-805, B-1487
P. reptilovora	B-2018, B-2021, B-2846
P. saccharophila	B-628
P. septica	B-1962
P. spe.	B-89

(continued)

TABLE 3 (continued)

Microorganisms	NRRL No.
Yeasts:	
Candida apicola	Y-2481
C. azyma	Y-17067
C. cacaoi	Y-7302
C. dendronema	Y-7781
C. freyschussii	Y-7898
C. humicola	Y-12944
C. magnoliae	Y-2333, YB-4226
C. maltosa	Y-5704
C. melinii	Y-1514, Y-1782, Y-2326
C. methylica	Y-17325
C. quercitrusa	Y-5392
Pichia bimundalis	Y-5343
P. holstii	Y-2155
P. lynferdii	Y-7723
P. membranaefaciens	Y-1513
P. mississippiensis	Y-11748
P. petersonii	YB-3808
P. silvicola	Y-1678
P. sydowiorum	Y-7130
Saccharomycopsis crataegensis	YB-192
Yarrowia lipolytica	YB-423, Y-2178
Zygosaccharomyces fermentati	Y-17056
Actinomycetes:	
Paecilomyces lilaciouis	13026, 2014, 895
Streptomyces aculentosporus	B-11397
S. capoamus	B-3632
Fungi:	
Penicillium citrinum	2140

TABLE 4

Microorganisms That Produce Orange Fluorescent Halo Zones Equal To and Larger Than 10 mm in Diameter

Microorganisms	Northern Regional Research Lab (NRRL) No.
Bacteria:	
Unknown species	B-3281
Bacillus amyloliquefaciens	B-207, B-448, B-1470, B-2613
B. magaterium	B-352, B-938, B-1370, B-1851, B-3254
B. pumilus	NRS-307, NRS-309, B-4064
B. subtilis	B-209, B-359, B-360, B-554
Pseudomonas aeruginosa	B-26, B-217, B-219, B-220, B-221, B-257, B-264, B-323, B-771, B-2019, B-2075, B-3464, B-3748
P. chlorophis	B-2019, B-2075
P. fluorescens	B-97, B-189, B-258, B-538 B-1799, B-1800, B-1882, B-2458
P. fragi	B-73, B-955, B-2271, B-2316
P. marginata	B-792
P. mucidolens	B-18
P. myxogenes	B-2086, B-2105, B-2106, B-2107 B-2108, B-2109
P. nonliquefaciens	B-1023
P. perolens	B-1123
P. reptilovora	B-6, B-712, B-713, B-1961, B-2017, B-2845, B-2847
P. riboflavina	B-949
P. septica	B-2081
P. species	B-915, B-964, B-965
P. stutzeri	B-775
P. tolaasii	B-991
P. viridilivida	B-1032
P. viscosa	B-1302

(continued)

TABLE 4 (continued)

Microorganisms	NRRL No.
Yeasts:	
Candida quercuum	Y-12942
C. silvanorum	Y-7782
C. silvicola	YB-2846
C. spe.	YB-2064
Geotrichum candidum	Y-552
Pichia americana	Y-2156, YB-2444
P. anomala	Y-993
P. candensis	Y-1888, Y-2340
P. holstii	Y-7914
P. muscicola	Y-7005, Y-7006
P. rabaulensis	Y-11533
Trichosporon species	YB-4547
Yarrowia lipolytica	Y-1095, Y-679
Actinomycetes:	
Nocardia aurantia	F-4209, B-3287
Streptomyces afghaniensis	B-5621
S. albogriseolus	B-16393
S. aureus	B-16044, B-1941
Fungi:	
Penicillium citrinum	3754, 5907
P. funiculosum	6014

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