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pH dependence and thermostability of lipases from cultures from the ARS Culture Collection

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SUMMARY

Previously we used a simple, sensitive agar plate method 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 were lipase-positive. These lipase-positive strains were further classified as good, moderate or weak enzyme producers. We have expanded our screening method to focus specifically on the pH dependence and thermostability of these lipase activities. The lipases exhibited various pH sensitivities and were divided into three groups: (i) lipases which are active at pH 5.5 but not at pH 7.5—produced by 36 bacteria, 23 yeasts and four actinomycetes; (ii) lipases which are active at pH 7.5 but not at pH 5.5—produced by 17 bacteria, four yeasts, two actinomycetes and one fungus; and (iii) lipases which are active at both pH 5.5 and pH 7.5—produced by 112 bacteria, 90 yeasts, 15 actinomycetes and five fungi. By screening at 60 °C and pH 9.0, we further identified 50 bacteria and 26 yeasts that produce thermostable alkali-tolerant lipases. Product analyses confirmed our screening results. Lipases with specific pH dependency and thermostability have potential to be developed into industrial enzymes.

INTRODUCTION

Lipases (3.1.1.3) catalyze the hydrolysis of triglycerides or acyl and aryl esters. They also are capable of catalyzing organic reactions in non-aqueous media [5]. Large quantities of microbial lipases are being produced on an industrial scale for medical and industrial uses. Lipases hydrolyze or synthesize triglycerides with positional and fatty acids specificities. For example, lipases from *Aspergillus niger* and *Rhizopus delemar* hydrolyzed/formed ester bonds only at positions 1 and 3 of glycerol [10]. In contrast, lipases from *Geotricum candidum* and *Penicillium cyclopium* hydrolyze or form ester bonds at all three positions [10]. For industrial hydrolysis, it is better that the reactants and products be liquid, and with many oils and fats a reaction temperature greater than 60 °C is required. Unfortunately, lipases that are both stable at greater than 60 °C and capable of extensive fat splitting are not available. The most effective lipase from *Candida rugosa* is inactivated at temperatures greater than 40 °C. In general, immobilized lipase increases enzyme thermostability. Lipase-catalyzed esterification processes have been commercialized by Unichema International (Gouda, The Netherlands) [2] for the production of high quality fatty esters, such as isopropylmyristate, isopropylpalmitate and 2-ethylhexylpalmitate, which are used as ingredients in skin creams and other personal care products. In these processes, the reactants are stirred at 50–70 °C in non-aqueous media with lipase immobilized on macroporous particles.

In recent years, interest in the use of enzymes as hydrolytic or synthetic chiral catalysts has risen rapidly. Extracellular microbial lipases are particularly suited for this application. Because of their availability, more than one dozen commercial lipases have been studied extensively by researchers in both industry and academe. For example, Sih et al. [9] studied several commercial lipases for the enantiospecific hydrolysis of 2-arylpropionic acid esters, an important class of nonsteroidal antiinflammatory drugs, and the aryl-thio-2*S*-methyl-propionic acid esters, key chiral intermediates for the synthesis of the antihypertensive agent captopril. Barton et al. [1] studied several commercial lipases for enzymatic resolution of phenoxy compounds such as (*R*,*S*)-2-(4-hydroxyphenoxy)propionic acids, valuable as intermediates in the production of certain herbicides. However, under most conditions, the enantioselectivity has not been high enough to permit economically useful production of the desired enantiomer.

Industry continues to look for economical sources of lipases and esterases with high activities. New lipases from microbial sources have been reported sporadically [6,8,11]. The need for novel lipases and esterases is obvious, but to our knowledge, no effort has been made or is being planned for conducting a large scale systematic screening for new lipases/esterases. At the National Center for Agricultural Utilization Research (NCAUR, Peoria, IL, USA) with the support of the Biotechnology Research and Development Corporation (Peoria, IL, USA), we had the opportunity to

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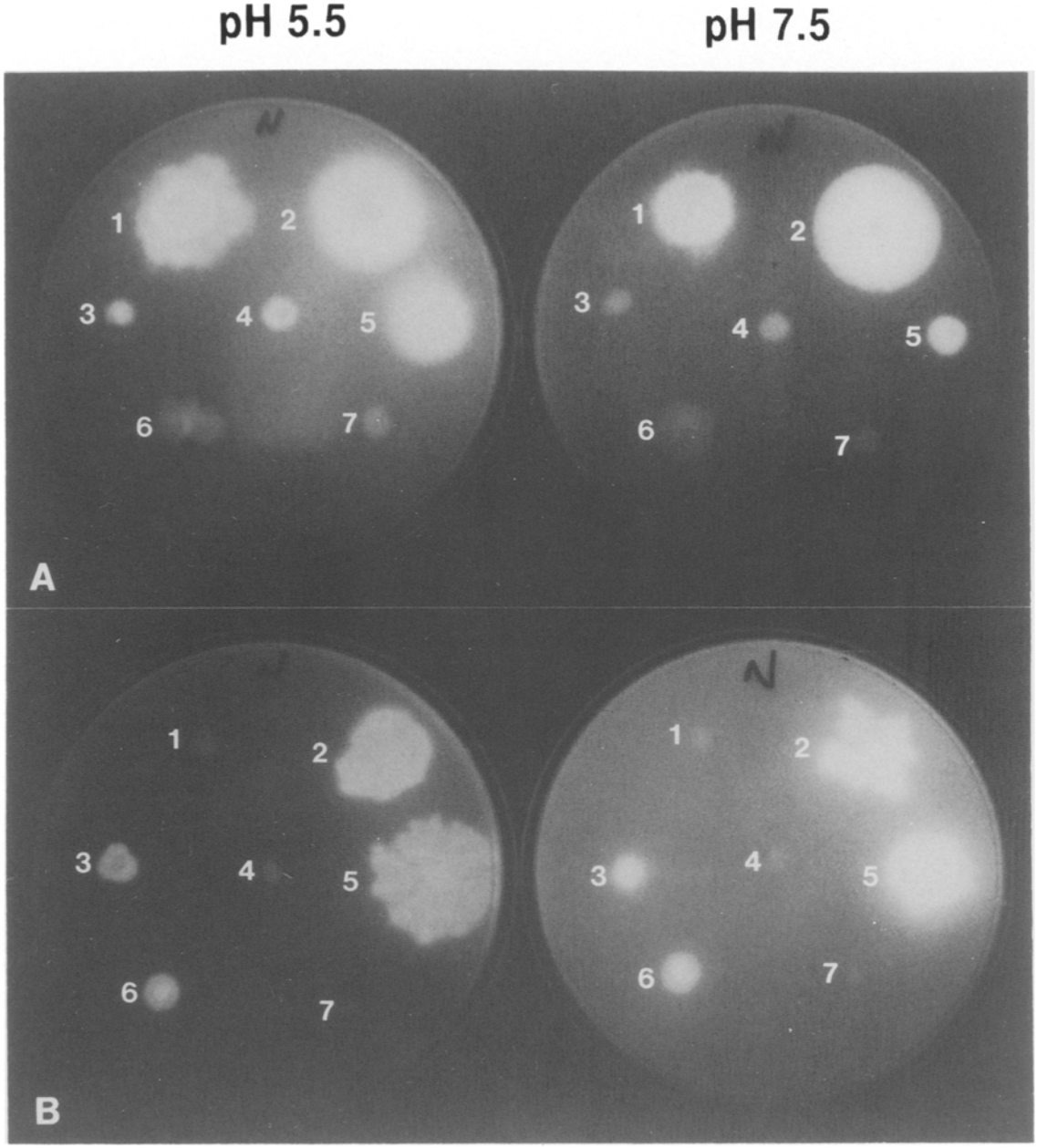

Fig. 1. Rhodamine B agar plate screen for lipase activity. (A) Yeasts: (1) *Yarrowia lipolytica* NRRL Y-1095; (2) *Geotricum candidum* NRRL Y-552; (3) *Candida magnoliae* NRRL Y-2024; (4) *C. magnoliae* Y-4226; (5) *C. azyma* NRRL Y-17067; (6) *C. krusei* NRRL Y-17013; (7) *C. lambica* NRRL Y-100. (B) 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; (7) *Ps. putida* NRRL B-2024.

conduct a large scale screening for lipase and esterase activity with cultures in the ARS Culture Collection (Peoria, IL, USA).

We previously used a modified rhodamine B agar plate method [4,7] to screen lipase activity in 1229 selected cultures covering many genera and species of bacteria, yeasts, actinomycetes, and fungi. About 25% of the cultures screened were lipase-positive. Some of these lipases also function as esterases, stereospecifically hydrolyzing 2-ethylhexyl butyrate to produce *S*(+)2-ethylhexanol [3]. Now we have expanded our screening methods to focus specifically on the pH dependence and thermostability of these lipase activities. Surprisingly, we found many organisms that express lipase activity only under acidic, neutral or alkaline conditions. Many thermostable alkali-tolerant lipases also were identified. This paper presents the results of our screening operation.

TABLE 1

Microorganisms that produce lipases active at pH 5.5 but not at pH 7.5

Microorganisms	NRRL number
YEASTS	
Candida ancudensis	Y-17327
Candida antarctica	Y-7954
Candida atmospherica	Y-5979
Candida bombi	Y-17081
Candida buffonii	Y-17082
Candida cacaoi	Y-7302
Candida chilensis	Y-17141
Candida geochares	Y-17073
Candida lipolytica	Y-2178
Candida magnoliae	Y-2024, Y-2333, YB-4226, Y-7621, Y-7622
Candida maritima	Y-7899
Candida salmanticensis	Y-17090
Candida savonica	Y-17077
Pichia glucozyma	YB-2185
Pichia musicola	Y-7006
Pichia petersonii	YB-3808
Pichia silvicola	Y-1678
Pichia sydowiorum	Y-7130
Saccharomycopsis fibuligera	Y-12677
ACTINOMYCETES	
Unknown name	B-12699
Chainia purpurogena	B-2952
Streptomyces aureus	B-16044
Streptomyces flavovirens	B-2685
BACTERIA	
Alcaligenes faecalis	B-1695
Bacillus amyloliquefaciens	B-207
Bacillus megaterium	B-1827, B-1851, B-352, B-47
Bacillus subtilis	B-554
Pseudomonas acidovorans	B-980
Pseudomonas aeruginosa	B-23, B-248, B-79, B-27
Pseudomonas chlororaphis	B-1869, B-2075
Pseudomonas fluorescens	B-1608, B-1897, B-258, B-2640, B-97
Pseudomonas fragi	B-955
Pseudomonas myxogenes	B-2108
Pseudomonas putida	B-1245, B-13, B-2023, B-2174, B-2336, B-254, B-805, B-931, B-2079, B-8
Pseudomonas putrifaciens	B-9517
Pseudomonas reptilovora	B-6, B-712
Pseudomonas syncyanea	B-1246
Pseudomonas viscosa	B-2538

TABLE 2

Microorganisms that produce lipases active at pH 7.5 but not at pH 5.5

Microorganisms	NRRL number
YEASTS	
Pichia alni	Y-11625
Pichia membranaefaciens	Y-1513
Pichia meyerae	Y-12777
Saccharomycopsis crataegensis	YB-192
BACTERIA	
Altermonas spp.	B-956, B-973
Bacillus amyloliquefaciens	B-1466, B-2613
Bacillus circulans	B-383
Bacillus megaterium	B-938
Pseudomonas aeruginosa	B-221
Pseudomonas chlororaphis	B-1541, B-1632
Pseudomonas fragi	B-2316, B-73
Pseudomonas myxogenes	B-2105
Pseudomonas perolens	B-1123
Pseudomonas reptilovora	B-1961
Pseudomonas septica	B-1963, B-2082
Pseudomonas stutzeri	B-775
ACTINOMYCETES	
Rhodococcus rhodochrous	B-16562
Streptomyces albus	B-2380
FUNGUS	
Penicillium citrinum	6336

MATERIALS AND METHODS

Microorganisms

All microbial cultures were obtained from the ARS Culture Collection at NCAUR. Bacteria were grown at 30 °C and pH 5.5, 7.5 or 9.0 on TGY medium which contained (per liter): tryptone, 5 g; yeast extract, 5 g; dextrose, 1 g; K_2HPO_4, 1 g. Yeasts, actinomycetes, and fungi were grown at 25 °C and at pH 5.5, 7.5 or 9.0 on PDA medium which contained (per liter): 26 g PDA (Difco Lab, Detroit, MI, USA). For preparing agar plates, 10 g agar was added into the above mentioned media.

Chemicals

Commercial lipases (triacylglycerol lipases, EC 3.1.1.3) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Rhodamine B and soybean oil were purchased from Sigma Chemical Co. Thin-layer precoated Kieselgel 60 F_{254} plates were obtained from EM Science (Cherry Hill, NJ, USA). All other chemicals were reagent grade and were used without further purification.

Lipase activity screen

Media were autoclaved and cooled to about 60 °C, after which for each liter of medium, 30 ml of filter-sterilized soybean oil and 2 ml of filter-sterilized aqueous solution of rhodamine B (0.1%) were added with vigorous shaking. After the agar medium was allowed to stand for 10 min at 60 °C to reduce foaming, 20 ml of the medium was poured into sterile petri plates. Cultures were inoculated from an agar slant as a small spot on the screening agar plate and incubated at 30 °C for bacteria and 25 °C for yeasts,

TABLE 3

Microorganisms that produce lipases active at both pH 5.5 and 7.5

Microorganisms	NRRL number
YEASTS	
Candida apicola	Y-2481, Y-5391
Candida azyma	Y-17067
Candida boleticola	Y-17080
Candida bombicola	Y-17069
Candida dendrica	Y-7775
Candida entomaea	Y-7785
Candida entomophila	Y-7783
Candida ernobii	Y-12941
Candida freyschussi	Y-7898
Candida fusiformata	Y-17173
Candida hellenica	Y-17319, Y-6591
Candida humicola	Y-1266, Y-12944
Candida homilentoma	Y-10941
Candida insectamans	Y-7786
Candida kruisii	Y-17086
Candida lipolytica	Y-6795
Candida lusitaniae	Y-7940
Candida magnoliae	Y-680
Candida maltosa	Y-5704
Candida melinii	Y-1514, Y-1782, Y-2326
Candida methylica	Y-17325
Candida mucilagina	Y-11823
Candida naeodendra	Y-10942
Candida nodaensis	Y-2484
Candida norvegica	Y-6700
Candida oregonensis	Y-5850
Candida parapsilosis	Y-7629, Y-7659
Candida philyla	Y-7776
Candida quercitrusa	Y-5392
Candida quercuum	Y-12942
Candida santamariae	Y-6656
Candida silvanorum	Y-7782
Candida silvicola	Y-6052, YB-2846
Candida silvicultrix	Y-7789
Candida spp.	Y-490, YB-2064, YB-599
Candida tanzawaensis	Y-17324
Geotrichum candidum	Y-552
Pichia abadieae	Y-7499
Pichia americana	Y-2156, YB-2444
Pichia anomala	Y-366, Y-993
Pichia bimundalis	Y-5343, YB-2805
Pichia bispora	Y-1482, Y-11610
Pichia canadensis	Y-1888, Y-2340
Pichia dryadoides	Y-10990
Pichia euphorbiophila	Y-12742, Y-12743
Pichia fabianii	Y-1871, Y-1873
Pichia hampshirensis	YB-4128
Pichia holstii	Y-2155, Y-7914
Pichia insitovora	Y-12698
Pichia lynferdii	Y-7723
Pichia mexicana	Y-11818, Y-11819
Pichia minuta	Y-10948
Pichia mississippiensis	Y-11748, YB-1294
Pichia muscicola	Y-7005
Pichia nakazawae	Y-7904
Pichia rabaulensis	Y-11533
Pichia silvicola	YB-3086
Pichia toletana	YB-4247
Pichia veronae	Y-7818
Pichia xylosa	Y-12929, Y-12939, Y-5987, YB-3884, YB-3887
Saccharomycopsis crataegensis	Y-5902
Saccharomycopsis lipolytica	YB-423
Trichosporon spp.	Y-1489, YB-4547
Wickerhamiella domercqiae	Y-6698
Yarrowia lipolytica	Y-1095
Zygosaccharomyces fermentati	Y-17056
ACTINOMYCETES	
Unknown name	B-7659, F-4209
Chainia poonensis	B-2319
Chainia violens	B-3483
Geosmithia ladendula	B-13026, B-2014, B-895
Nocardia gibsonii	B-2592
Streptomyces aculentosporus	B-11397
Streptomyces afghaniensis	B-5621
Streptomyces albogriseolus	B-16393
Streptomyces aureus	B-1941
Streptomyces capoamus	B-3632
Streptomyces carneus	B-2001
Streptomyces griseus	B-2027
FUNGI	
Penicillium citrinum	2140, 3754, 5907
Penicillium funiculosum	13095, 6014
BACTERIA	
Arthrobacter terregens	B-14092
Bacillus amyloliquefaciens	B-1470, B-448
Bacillus megaterium	B-1367, B-1370, B-3254
Bacillus pumilus	B-208, B-3480, B-3481, N-309, B-307, N-307
Bacillus subtilus	B-359, B-361, B-364, B-209, B-360, B-542
Pseudomonas aeruginosa	B-12, B-184, B-1852, B-211, B-217, B-219, B-220, B-247, B-249, B-250, B-256, B-257, B-26, B-264, B-265, B-275, B-282, B-323, B-3464, B-3509, B-3748, B-4014, B-450, B-451, B-452, B-534, B-7, B-771
Pseudomonas acidovorans	B-979
Pseudomonas antimycetica	B-1683
Pseudomonas chlororaphis	B-1097, B-1870, B-2019, B-560, B-2266, B-561
Pseudomonas fluorescens	B-1105, B-1244, B-1603, B-1609, B-1612, B-1636, B-1796, B-1799, B-1800, B-1882, B-189, B-1944, B-1964, B-2322, B-2458, B-2547, B-4290, B-538, B-804
Pseudomonas fragi	B-2771
Pseudomonas marginata	B-955
Pseudomonas mexicana	B-4222
Pseudomonas mucidolens	B-18
Pseudomonas myxogenes	B-2086, B-2106, B-2107, B-2109
Pseudomonas nonliquefaciens	B-1023, B-993
Pseudomonas pavonacea	B-724
Pseudomonas putida	B-1487
Pseudomonas reptilovora	B-2017, B-2018, B-2021, B-2845, B-2846, B-2847, B-713, B-81, B-89, B-964, B-965
Pseudomonas resinovorans	B-2649
Pseudomonas riboflavina	B-949
Pseudomonas saccharophila	B-628
Pseudomonas seminum	B-2742
Pseudomonas septica	B-2081, B-1962
Pseudomonas spp.	B-1878, B-1880, B-1794, B-1881
Pseudomonas suis	B-915, B-927
Pseudomonas tolaasii	B-991
Pseudomonas viridilivida	B-893
Pseudomonas viscosa	B-1302
Unknown name	B-3281

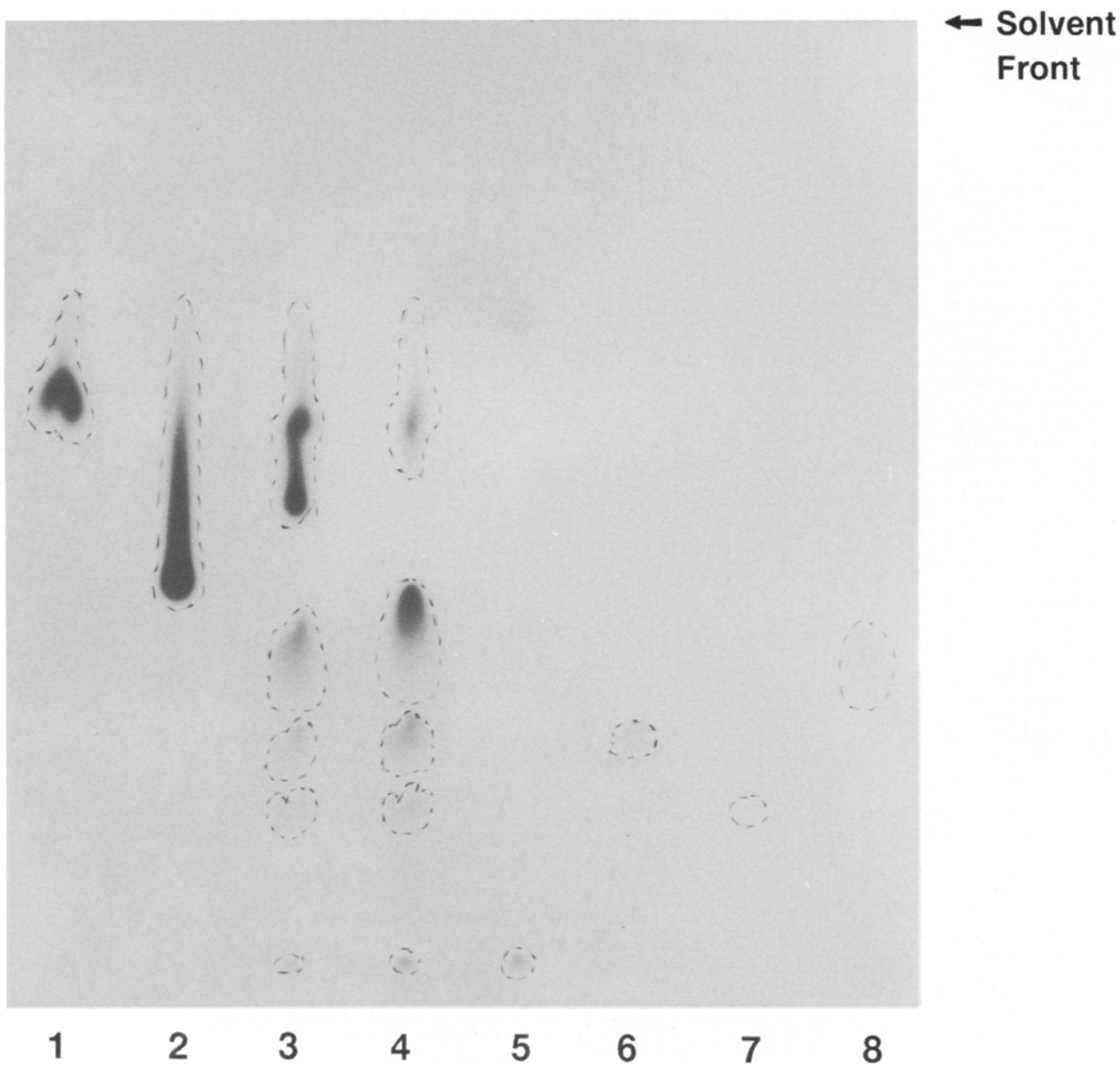

Fig. 2. Thin-layer chromatography of lipase hydrolysis products. Two-stage development procedure in one dimension: (A) benzene, ether, ethyl acetate, acetic acid (80:10:10:1 v/v/v/v); (B) hexane, ether, formic acid (80:20:2 v/v/v). Spots with dotted lines were visualized with iodine vapor. The spot in lane 7 showed as a white spot over a brownish background. (1) soybean oil; (2) soybean oil plus a lipase-negative strain (*Pseudomonas acidovorans* NRRL B-873); (3) soybean oil plus a Sigma lipase (type II from porcine pancreas); (4) soybean oil plus a lipase-positive strain (*Pseudomonas myxogenes* NRRL B-2106); (5) 1-mono-oleoyl-rac-glycerol; (6) 1,3-diolein; (7) 1,2-distearyl-rac-glycerol; (8) oleic acid.

actinomycetes, and fungi. Lipase activity was identified on the plate as an orange fluorescent halo around the colonies after 48 h of incubation. As a control, various quantities of a commercial pancreatic lipase were assayed with this screening plate. Sizes of their orange fluorescent halo correlated well with lipase activities [4]. For screening at 60 °C and pH 9.0, a few drops of sterile water were added to the inside of the petri cover plate. The inner plate with agar medium was placed on top of the cover plate to reduce drying of the agar medium. The petri plates also were sealed with tape to further prevent the drying process during incubation.

Identification of lipase hydrolysis products

A 1-g portion of filter-sterilized soybean oil was added to a 1-day-old culture of either a lipase-negative strain

TABLE 4

Microorganisms that produce lipases active at pH 9 at 60 °C

Microorganisms	NRRL number
YEASTS	
Candida fennica	Y-7505
Candida rugopelliculosa	Y-17079
Candida sake	YB-1132
Candida silvicola	YB-2846, Y-6052
Candida sorbophila	Y-7921
Candida utilis	Y-7586
Issatchenkia orientalis	Y-7550
Pichia abadieae	Y-7499
Pichia alni	Y-11627
Pichia fabianii	Y-1873, Y-1871
Pichia guilliermondii	Y-2075
Pichia hampshirensis	YB-4128
Pichia inositovora	Y-12698
Pichia mississippiensis	YB-1294
Pichia nakazawae	Y-7904
Pichia rabaulensis	Y-11533
Pichia silvicola	YB-3086
Pichia xylosa	Y-12939
Saccharomycopsis lipolytica	YB-423
Torulaspora globosa	Y-1982
Williopsis pratensis	Y-12696
Zygosaccharomyces bailii	Y-7260, Y-7258
Zygosaccharomyces rouxii	Y-998
BACTERIA	
Altermonas sp.	B-808
Bacillus brevis	NRS-603
Bacillus firmus	NRS-854
Bacillus licheniformis	NRS-12648
Bacillus pumilus	B-14292, N-331
Bacterium indoloxidans	B-1038
Leuconostoc dextranicum	B-1420
Micrococcus luteus	B-1106, B-4375
Micrococcus spp.	B-1018, B-476, B-555
Pseudomonas acidovorans	B-802
Pseudomonas aeruginosa	B-184, B-211, B-218, B-219, B-221, B-23, B-248, B-255, B-256, B-257, B-264, B-265, B-27, B-282, B-323, B-325, B-771
Pseudomonas chlororaphis	B-2019, B-977
Pseudomonas fluorescens	B-11, B-1799, B-804
Pseudomonas fragi	B-727
Pseudomonas mephitica	B-75
Pseudomonas mucidolen	B-18
Pseudomonas myxogenes	B-2105, B-2106, B-2107
Pseudomonas nonliquefaciens	B-995
Pseudomonas reptilovora	B-2017, B-713, B-81
Pseudomonas ribis	B-160
Pseudomonas riboflavina	B-949
Pseudomonas viridilivida	B-893, B-895

(*Pseudomonas acidovorans* NRRL B-783) or a lipase-positive strain (*Pseudomonas myxogenes* NRRL B-2106) grown on 30 ml TGY medium at 30 °C and incubation was continued for an additional three days. In the case of commercially available lipase, 100 lipase units of Sigma lipase II (from porcine pancreas) were added to 10 ml 0.05 M sodium phosphate buffer solution (pH 7.5) containing 0.3 g soybean oil and incubated at 37 °C for 24 h. At the end of the incubation, the reaction mixture was acidified with dilute HCl to pH 2 and extracted twice with an equal volume of diethyl ether. The ether extract was concentrated using a rotary evaporator and analyzed by TLC. TLC plates were developed with a two-stage development procedure: (A) benzene, ether, ethyl acetate, acetic acid (80:10:10:1, v/v/v/v), development finished when solvent was 10 cm above the origin; (B) hexane, ether, formic acid (80:20:2, v/v/v), development in the same direction to top of the TLC plate. After development, products on the plate were identified first by exposure to iodine vapor and then by spraying with 60% aqueous sulfuric acid and charring.

RESULTS

Typical results of lipase screening operations on yeasts and bacteria are shown in Fig. 1(A) and 1(B), respectively. It is easy to identify the orange fluorescent haloes characteristic of lipase activity and thereby identify strains producing lipases. A total of 1229 cultures including 508 bacteria, 479 yeasts, 230 actinomycetes and 12 fungi were screened at pH 5.5, 7.5 and 9.0. The genera and species of these cultures were reported previously [4].

Among the 1229 cultures screened, 168 bacteria, 119 yeasts, 22 actinomycetes and six fungi were lipase-positive. The lipases from these positive strains behaved differently in different pH environments. As listed in Table 1, lipases from 36 bacteria, 23 yeasts and four actinomycetes were active at pH 5.5 and inactive at pH 7.5. Lipases from 17 bacteria, four yeasts, two actinomycetes and one fungus were active at pH 7.5 but not at pH 5.5 (Table 2). One hundred and twelve bacteria, 90 yeasts, 15 actinomycetes and five fungi produced lipase activity at both pH 5.5 and 7.5 (Table 3).

Lipase activity was confirmed by analyzing the products of incubation of soybean oil with a lipase-positive organism. When soybean oil was incubated with the lipase-positive strain, *Pseudomonas myxogenes* NRRL B-2106, hydrolysis products were generated consisting of diglycerides(1,2- and 1,3-), monoglycerides, and free fatty acids similar to those obtained by commercially available lipase (Fig. 2). The lipase-negative strain, *Pseudomonas acidovorans* NRRL B-783, did not hydrolyze soybean oil. Thus, our screening method was correct in identifying lipase-producing strains.

Since there is industrial need for thermostable alkaline lipases, we also conducted our screen at 60 °C with the pH of the screening media at 9.0. Many cultures cannot grow under these conditions. However, among those grown, as listed in Table 4, 50 bacteria and 26 yeasts produced thermostable alkali-tolerant lipases. Classification of these

lipases according to their positional-, fatty acid- or chiral specificities will require further studies. Because the applications of lipases vary, further classification of these lipases must be left to the individual scientist. Nevertheless, the data presented here provide important information regarding previously unknown sources of lipases which might lead to discovery of new lipases suitable for industrial applications.

DISCUSSION

Lipases are important biocatalysts in biochemical reactions. In industry, the selection of the most appropriate lipase for a particular use is a vital concern and the existence of numerous microbial lipases encourages us that one may find the proper lipase for a given purpose. This is an additional reason why microorganisms are much more preferable as lipase sources than are animals or plants. The needs for novel lipases are obvious. We have screened 1229 cultures covering many genera and species in yeasts, bacteria, actinomycetes, and fungi. About 25% of these cultures possess lipase activity. These lipase activities were further identified as enzymes which were active at pH 5.5, at pH 7.5, or at both conditions. We also conducted our screen at 60 °C, pH 9.0 to identify thermostable alkali-tolerant lipases, and 50 strains of bacteria and 26 strains of yeasts showed lipase activity under these conditions. However, we are not able to distinguish whether the lipolytic activity detected here is due to pH-dependent lipase synthesis or pH-dependent lipase activity. It also is possible that this activity is due to synthesis of multiple lipases and our methods cannot differentiate single from multiple lipase synthesis. Product analyses confirmed our screening results (Fig. 2). Our data provide important first step information for the selection of a proper lipase for a given purpose. Screening of microorganisms, with almost limitless forms existing in nature, offers a fertile approach for discovering novel lipases.

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REFERENCES

1 Barton, M.J., J.P. Hamman, K.C. Fichter and G.J. Calton. 1990. Enzymic resolution of (*R,S*)-2-(4-hydroxyphenoxy)propionic acid. Enzyme Microbiol. Technol. 12: 577–583.

2 Casy, J. and A. Macrae. 1992. Biotechnology and the oleochemical industry. Inform 3: 203–207.

3 Hou, C.T. 1993. Screening of microbial enzyme for asymmetric hydrolysis of 2-ethylhexyl butyrate. J. Ind. Microbiol. 11: 73–81.

4 Hou, C. T. and T. Johnston. 1992. Screening of lipase activities with cultures from ARS Culture Collection. J. Am. Oil Chem. Soc. 69: 1088–1097.

5 Klibanov, A.M. 1986. Enzymes that work in organic solvent. Chem. Technol. 16: 354–359.

6 Kokusho, Y., H. Machida and S. Iwasaki. 1982. Studies on alkaline lipase: isolation and identification of lipase producing microorganisms. Agric. Biol. Chem. 46: 1159–1164.

7 Kouker, G. and K.E. Jaeger. 1987. Specific and sensitive plate assay for bacterial lipases. Appl. Environ. Microbiol. 53: 211–213.

8 Machida, H., T. Higashi and Y. Kokusho. 1984. Industrial production and application of microbial lipases. Nippon Nogei Kagaku Kaishi 58: 799–804.

9 Sih, C.J., Q.-M. Gu, G. Fulling, S.-H. Wu and D.R. Reddy. 1988. The use of microbial enzymes for the synthesis of optically active pharmaceuticals. Dev. Ind. Microbiol. 29: 221–229.

10 Tsujisaka, Y. and M. Iwai. 1984. Comparative study on microbial lipases. Kagaku To Kogyo 58: 60–69.

11 Wang, Y.-X., K.C. Srivastava, G.-J. Shen and H.Y. Wang. 1991. Novel thermostable alkaline lipase from a new thermophilic *Bacillus* strain A30-1. Abstract F8. Am. Oil Chem. Soc. Annual Meeting, Chicago, IL, USA.