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Screening of microbial esterases for asymmetric hydrolysis of 2-ethylhexyl butyrate

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SUMMARY

A pH indicator agar plate method was used to screen for esterase activities for hydrolysis of 2-ethylhexyl butyrate. Seven hundred and fifty-seven selected microbial cultures, including 325 bacteria and 432 yeasts and actinomycetes from the ARS Culture Collection, were screened. Among them, 62 cultures hydrolyzed 2-ethylhexyl butyrate. Of these strains only 17 showed lipase activity on a rhodamine B lipase screen. The reaction products, 2-ethyl-1-hexanol and *n*-butyric acid, were confirmed by gas-liquid chromatography (GC) and GC/MS analyses. The yield of 2-ethyl-1-hexanol varied depending on the strains of the microorganisms, with the highest yield at 79.1% by a strain of *Pseudomonas myxogenes*. Product analyses with a cyclodextrin GC chiral column showed that two strains of *Pseudomonas* produced greater than 80% enantiomeric excess of S(+)-2-ethyl-1-hexanol.

INTRODUCTION

Esterases (EC 3.1.1.1. and 3.1.1.2) are different from lipases (EC 3.1.1.3.) in that they cannot hydrolyze triacylglycerols. Lipolytic activity toward organic esters differs from enzyme to enzyme depending on the origin of the enzyme. Stereospecific esters can be synthesized from alcohols or acids by lipases or esterases [4,5]. There are many useful chiral specific acids, alcohols, and esters, e.g., naproxen, ketoprofen and many intermediates for the synthesis of important drugs, insecticides and herbicides. Previously, we used a rhodamine B agar plate method to screen for lipase activity from 1229 selected cultures including bacteria, yeasts, actinomycetes, and filamentous fungi covering many genera and species [3]. Lipolytic activity was found in many species not previously known to produce lipase. Lipases are known to hydrolyze triglycerides with positional and fatty acid specificity [8]. Similar to esterases they also perform asymmetric hydrolysis of organic esters [6]. In this paper we are interested in screening enzymes from microorganisms for asymmetric hydrolysis of 2-ethylhexyl butyrate to produce stereospecific enantiomers of 2-ethyl-1-hexanol. 2-Ethylhexyl butyrate was selected as the substrate, because it contains a structural feature representative of that found in many enantiomeric chemical targets. We used a pH indicator agar plate method to screen 757 cultures, selected from the ARS Culture Collection, for their ability to hydrolyze 2-ethylhexyl butyrate. Sixty-two cultures were positive. The reaction products were identified by gas-liquid chromatography (GC) and GC/MS analyses. Some strains preferentially hydrolyzed one enantiomer. This paper describes our screening results.

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MATERIALS AND METHODS

Microorganisms

All microbial cultures were obtained from the ARS Culture Collection (Peoria, IL). Bacteria were grown on TGY medium which contained (per liter): tryptone, 5 g, yeast extract 5 g, dextrose 1 g, K_2HPO_4 1 g, pH 7.3 at 30 °C. Yeasts and actinomycetes were grown on YM medium which contained (per liter): yeast extract 3 g, malt extract 3 g, peptone 5 g and dextrose 10 g, pH 7.0 at 25 °C. For preparing agar plates, 20 g agar was added into the above-mentioned media.

Chemicals

2-Ethylhexyl butyrate, 2-ethyl-1-hexanol, bromthymol blue, lipases (triacylglycerol lipases, EC 3.1.1.3) and es-

terases (EC 3.1.1.1) were purchased from Sigma Chemical Co. The lipases included: porcine pancreas lipase (type II), *Candida cylindracea* lipase (type VII), wheat germ lipase (type I), *Pseudomonas* spp. lipase (type XII), and *Rhizopus arrhizus* lipase (type XI). The esterases were: rabbit liver esterase and porcine liver esterase. All other chemicals were reagent grade and were used without further purification.

Enzyme activity screen

One hundred milliliters of media was autoclaved and allowed to cool to about 60 °C. Then, 600 μl of filter-sterilized 2-ethylhexyl butyrate and 7.5 ml of filter-sterilized bromthymol blue in H_2O (4 mg/10 ml) were added with vigorous shaking. After the medium was allowed to stand for 10 min at 60 °C to reduce foaming, 20 ml of the medium was poured into each plate. Cultures were inoculated from their agar slant as a small spot on the screening agar plate and incubated at 30 °C for bacteria and 25 °C for yeasts. Control plates, which were similar to the test plates except that they contained no 2-ethylhexyl butyrate, were run parallel to the test plates to rule out false positives due to the production of organic acids by some microorganisms. Enzyme activity was identified on the plates as a yellow zone after 48 h of incubation.

Microbial hydrolysis of 2-ethylhexyl butyrate

To confirm the 2-ethylhexyl butyrate hydrolysis activity, positive strains from the agar plate screen were grown in 125-ml flasks containing 30 ml of the respective media at 150 rpm for 2 days. After that, 600 μl of 2-ethylhexyl butyrate was added, and incubation continued for an additional 24 h. At the end of the incubation, the culture broth was acidified to pH 2 with 6 M hydrochloric acid. The culture broth was then extracted with an equal volume of diethyl ether. The solvent was removed from the extracts with a rotary evaporator.

Enzymic hydrolysis of 2-ethylhexyl butyrate by commercially available esterases or lipases was conducted as follows. One hundred and twenty-five units of either esterase or lipase were added to 5 ml 0.05 M sodium phosphate buffer (pH 7.5). To this mixture, 2-ethylhexyl butyrate (0.1 ml) was added. The reaction mixture was incubated at 37 °C (25 °C for esterases) and 250 rpm for 5 h. At the end of the reaction, the mixture was acidified and extracted with an equal volume of diethyl ether as described above.

Analysis of products

The isolated hydrolysis products were analyzed by gas-liquid chromatography. Samples were injected into a Hewlett Packard model 5890 gas chromatograph equipped with a flame ionization detector, a Supelco SPB-1™ capillary column 15 m, i.d. 0.32 mm, 0.25 μm thickness and a Hewlett Parkard 3392A integrator. GC was run with a temperature gradient starting from 70 °C and then increased 5 °C per min to 140 °C. A typical gas chromatogram is shown in Fig. 1A. The same gas chromatograph, equipped with a Supelco 20% β-cyclodextran capillary column 60 m, i.d. 0.25 mm, 0.25 μm thickness was used for chiral analyses. The chiral gas chromatography was run isothermally at 100 °C for 35 min and then increased to 150 °C to clean up the column. A typical chiral gas chromatogram is shown in Fig. 1B. Mass spectra were obtained with a Perkin-Elmer Sigma 3B Capillary GC coupled to a Hewlett Packard 5970 Series Mass Selective Detector. Optical rotation measurements were performed with a Perkin-Elmer 241 polarimeter.

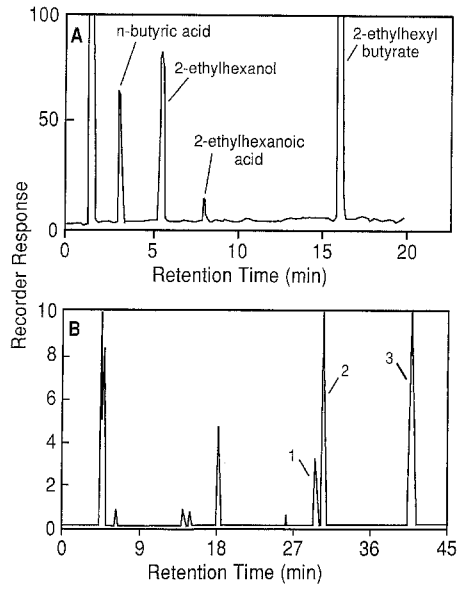

Fig. 1. (A) Gas chromatogram of 2-ethylhexyl butyrate hydrolysis products. GC conditions were as described in Materials and Methods. (B) Enantioanalysis of 2-ethylhexyl butyrate hydrolysis products. A Supelco β-cyclodextran capillary column was used with other conditions as described in Materials and Methods. 1, R(−)-2-ethyl-1-hexanol: 2, S(+)-2-ethyl-1-hexanol; 3, 2-ethylhexyl butyrate.

RESULTS AND DISCUSSION

Seven hundred and fifty-seven cultures including three hundred and twenty-five bacteria and four hundred and thirty-two yeasts and actinomycetes covering many genera and species were screened. The numbers of strains screened and found esterase positive are listed in Table 1. Positive strains, which hydrolyzed 2-ethylhexyl butyrate,

TABLE 1

Number of strains screened and positives on hydrolysis of 2-ethylhexyl butyrate

Microorganisms	Number of strains tested	Number of strains positive
Bacteria		
Aeromonas punctata	1	0
Alcaligenes faccalis	1	0
Altermonas putrefactions	1	0
Altermonas sp.	3	1 (B-808)
Arthrobacter simplex	1	0
Arthrobacter globiformis	1	0
Arthrobacter citreus	1	0
Bacillus amyloliquefaciens	6	1 (B-545)
Bacillus cereus	2	0
Bacillus circulans	1	0
Bacillus licheniformis	4	0
Bacillus macerans	1	0
Bacillus megaterium	6	2 (B-350, B-1827)
Bacillus polymyxa	2	1 (B-335)
Bacillus pumilus	1	0
Bacillus subtilis	3	2 (B-364, B-1466)
Bacterium indoloxida	1	0
Campylobacter fetus	1	0
Enterobacter aerogenes	2	1 (B-167)
Escherichia coli	2	0
Flavobacterium aurantiacum	1	1 (B-184)
Gluconobacter oxydans	2	0
Leuconostoc mesenteroides	1	0
Pseudomonas acidovorans	6	1 (B-936)
Pseudomonas aeruginosa	33	5 (B-23*, B-323*, B-275*, B-257*, B-2785)
Pseudomonas aminovorans	1	1 (B-934)
Pseudomonas antimycetica	1	1 (B-1683*)
Pseudomonas aromatica	3	1 (B-2173)
Pseudomonas boreopolis	2	0
Pseudomonas calcoaceticus	1	0
Pseudomonas cepacia	1	0
Pseudomonas chlororaphis	4	1 (B-1095)
Pseudomonas citrinellolis	1	0
Pseudomonas diminuta	1	0
Pseudomonas excibis	2	0
Pseudomonas fluorescens	24	4 (B-1104, B-1609, B-1612*, B-1636*)
Pseudomonas fragi	5	1 (B-2316*)
Pseudomonas gladioli	2	2 (B-851, B-823)
Pseudomonas indigofera	2	1 (B-2646)
Pseudomonas maculicola	1	0
Pseudomonas maltophila	1	1 (B-2337)
Pseudomonas marginata	2	2 (B-792*, B-849)
Pseudomonas mephetica	1	0
Pseudomonas mexicana	1	0
Pseudomonas mildenbergii	2	0
Pseudomonas mucidolens	3	1 (B-18*)
Pseudomonas myxogenes	3	1 (B-19)
Pseudomonas nonliquefaciens	3	1 (B-994)
Pseudomonas pantotropha	1	0
Pseudomonas pavonacea	3	0
Pseudomonas perolens	2	0
Pseudomonas pseudoalcaligenes	1	0
Pseudomonas putida	22	0
Pseudomonas putrefaciens	4	0
Pseudomonas reptilivora	12	0
Pseudomonas ribicola	3	1 (B-151)
Pseudomonas ribis	1	1 (B-160)
Pseudomonas riboflavina	3	0
Pseudomonas rubescens	4	0
Pseudomonas saccharophila	2	0
Pseudomonas echinoides	2	1 (B-3127)
Pseudomonas seminum	1	1 (B-2742)
Pseudomonas septica	4	1 (B-1963*)
Pseudomonas sp.	5	1 (B-109)
Pseudomonas striafaciens	2	0
Pseudomonas stutzeri	3	0
Pseudomonas suis	4	1 (B-919)
Pseudomonas syncyanea	4	1 (B-1246)
Pseudomonas syringae	1	1 (B-848)
Pseudomonas taetrolens	4	0
Pseudomonas testosteroni	1	0
Pseudomonas ureae	1	0
Pseudomonas viridiflava	2	0
Pseudomonas viridilivida	4	2 (B-721, B-1032)
Pseudomonas viscosa	2	0
Unidentified isolates	17	2 (B-1883, ADM No. 4)
Yeasts		
Candida acuta	1	0
Candida antartica	1	0
Candida apicola	2	0
Candida apis	1	0
Candida atmospherica	1	0
Candida auringiensis	2	0
Candida brindinii	1	0
Candida boleticola	1	0
Candida bombi	1	0
Candida bombicola	1	0
Candida buffonii	1	0
Candida cacaoi	1	0
Candida canterellii	2	0
Candida cariosilignicola	1	0

TABLE 1
(continued)

Microorganisms	Number of strains tested	Number of strains positive
Candida castellii	1	0
Candida chilensis	1	0
Candida culliculosa	1	0
Candida dendronema	1	0
Candida diddensiae	4	1 (Y-6971)
Candida diversa	1	0
Candida edax	1	0
Candida entomophila	1	0
Candida eremophila	1	0
Candida ernobii	2	0
Candida etchellsii	2	0
Candida ethaanolica	1	0
Candida famata	1	0
Candida fennica	1	1 (Y-7505)
Candida flavificians	1	0
Candida fluviatilis	3	0
Candida fragoriorum	1	0
Candida freyschussii	1	0
Candida fructus	1	0
Candida geochares	1	0
Candida glabrata	2	0
Candida glucosophila	1	0
Candida gropengiesseri	1	0
Candida guilliermondii	2	0
Candida hellenica	1	0
Candida humicola	1	1 (Y-17222)
Candida humilis	1	0
Candida hydrocarbofumarica	1	0
Candida hylophila	1	0
Candida incommunis	1	0
Candida ingens	2	1 (Y-7796)
Candida inositophila	1	0
Candida insectalens	1	0
Candida insectamans	1	0
Candida kefyr	1	0
Candida kruissii	2	0
Candida krusei	1	0
Candida lambica	3	0
Candida lipolytica	2	0
Candida lodderae	1	0
Candida lusitaniae	1	0
Candida magnoliae	6	0
Candida mannitofaciens	1	0
Candida maritima	1	1 (Y-7899*)
Candida melinii	4	0
Candida membranaefaciens	4	0
Candida mogii	2	0
Candida molischiana	1	0
Candida mucilagina	2	0
Candida navarrensis	1	0

TABLE 1
(continued)

Microorganisms	Number of strains tested	Number of strains positive
Candida nemodendra	1	0
Candida nitratophila	1	0
Candida norvegiea	1	0
Candida oleophila	2	0
Candida oregonensis	2	0
Candida pampelonensis	1	0
Candida parapsilosis	2	0
Candida philyla	1	0
Candida pseudointermedia	1	0
Candida ptarmiganii	1	0
Candida pulcherrima	1	0
Candida quercuum	1	0
Candida quilliermondii	1	0
Candida rhagii	4	0
Candida rugopelliculosa	1	0
Candida rugosa	1	0
Candida saitoana	1	0
Candida sake	8	0
Candida salmanticensis	1	0
Candida santamariae	1	0
Candida savonica	1	0
Candida schatavii	1	0
Candida shehatae	2	0
Candida silvanorum	2	0
Candida silvatica	1	0
Candida silvicola	1	0
Candida silvicultrix	1	0
Candida sonorensis	2	0
Candida sorbophila	1	0
Candida spandovensis	1	0
Candida sp.	1	0
Candida stellata	2	0
Candida succiphila	2	0
Candida tenuis	3	0
Candida tropicalis	1	0
Candida utilis	2	0
Candida vartiovarrai	1	0
Candida vini	1	0
Candida wickerhamii	2	0
Citeromyces matritensis	1	0
Geotrichum candidum	1	0
Issatchenkia orientalis	2	0
Issatchenkia scutulata	2	0
Issatchenkia terricola	1	0
Clawispora opuntiae	2	0
Loddermyces elongisporus	1	0
Pachysolen tannophilus	2	0
Pachytichospora transvaalensis	1	0
Pichia abadieae	1	0

TABLE 1

(continued)

Microorganisms	Number of strains tested	Number of strains positive
Pichia acaciae	2	0
Pichia alni	2	0
Pichia amenthionina	2	0
Pichia americana	2	0
Pichia amylophila	1	0
Pichia angophorae	1	0
Pichia angusta	4	0
Pichia anomala	2	1 (Y-993*)
Pichia antillensis	2	0
Pichia besseyi	1	0
Pichia bimondalis	2	0
Pichia bispora	2	0
Pichia bovis	2	0
Pichia burtonii	1	0
Pichia cactophila	1	0
Pichia canadensis	2	0
Pichia capsulata	1	0
Pichia carsonii	2	0
Pichia castillae	1	1 (Y-7501)
Pichia cellobiosa	2	0
Pichia chambardii	1	0
Pichia ciferri	1	0
Pichia delftensis	1	0
Pichia deserticola	3	0
Pichia dispora	2	0
Pichia drydoides	2	0
Pichia etchellsii	2	0
Pichia euphorbiophila	2	0
Pichia fabianii	2	0
Pichia farinosa	2	1 (Y-2060)
Pichia fermentans	1	0
Pichia finlandica	1	0
Pichia fluxuum	1	0
Pichia glucozyma	2	0
Pichia guilliermondii	2	0
Pichia hampshirensis	1	0
Pichia halophila	2	0
Pichia heedii	2	0
Pichia henricii	2	0
Pichia holstii	2	0
Pichia inositovora	1	1 (Y-12698*)
Pichia jadinii	1	0
Pichia japonica	2	0
Pichia lynferdii	1	0
Pichia media	2	0
Pichia membranaefaciens	2	0
Pichia methanolica	1	0
Pichia mexicana	2	0
Pichia meyerae	1	0
Pichia minuta	3	0

TABLE 1

(continued)

Microorganisms	Number of strains tested	Number of strains positive
Pichia mississippiensis	1	0
Pichia muscicola	1	0
Pichia naganishii	1	0
Pichia nakasei	1	0
Pichia nakazawae	2	0
Pichia norvegensis	1	0
Pichia onychis	2	0
Pichia opuntiae	4	0
Pichia pastoris	2	0
Pichia petersonii	1	0
Pichia pinus	2	0
Pichia populi	1	0
Pichia quercum	1	0
Pichia rabaulensis	1	0
Pichia rhodanensis	2	0
Pichia saitoi	3	0
Pichia salictaria	2	0
Pichia sargentensis	1	0
Pichia scolyti	1	0
Pichia segobiensis	2	0
Pichia silvicola	2	0
Pichia sorbitophila	1	0
Pichia spartinae	1	0
Pichia stipitis	2	0
Pichia strasburgensis	2	0
Pichia subpelliculosa	2	0
Pichia sydowiorum	1	0
Pichia toletana	1	0
Pichia trehalophila	2	0
Pichia veronae	1	0
Pichia wickerhamii	2	0
Pichia xylosa	3	0
Pichia zaruensis	2	0
Saccharomyces cerevisiae	1	0
Saccharomycopsis capsularis	2	0
Saccharomycopsis crataegensis	2	0
Saccharomycopsis fibuligera	2	0
Saccharomycopsis malanga	1	0
Saccharomycopsis synnaedendra	1	0
Torulaspora delbrueckii	2	0
Torulaspora globosa	1	0
Torulaspora pretoriensis	1	0
Wickerhamiella domerqiae	1	0
Williopsis beijerinkii	2	0
Williopsis dimennae	2	0
Williopsis mrakii	2	0
Williopsis pratensis	1	0
Williopsis salicorniae	1	0

TABLE 1

(continued)

Microorganisms	Number of strains tested	Number of strains positive
Williopsis saturnus	3	0
Williopsis sp.	1	0
Wingea robertsii	1	0
Yarrowia lipolytica	1	0
Zygosaccharomyces microellipsoides	4	0
Zygosaccharomyces bailii	11	0
Zygosaccharomyces bisporus	2	1 (Y-12626)
Zygosaccharomyces cidri	4	0
Zygosaccharomyces fermentati	6	0
Zygosaccharomyces florentinus	1	0
Zygosaccharomyces rouxii	11	0
Unidentified isolates	56	3 (YB-2877)
Actinomycetes and fungi		
Chainia antibiotica	1	0
Chainia aurea	1	0
Chainia flava	1	0
Chainia fumigata	1	0
Chainia kunmingensis	1	0
Chainia nigra	1	0
Chainia ochracea	1	0
Chainia olivacea	1	0
Chainia poonensis	1	0
Chainia purpurogenea	2	1 (B-2952*)
Chainia rubra	1	0
Chainia violens	1	1 (B-3483*)
Elytorosporangium brasiliense	1	0
Nocardia gibsonii	2	0
Nocardia sp.	1	1 (B-16034)
Serratia marcescens	1	0
Streptomyces griseus	2	0
Streptomyces afghaniensis	1	0
Streptomyces agglomeratus	3	0
Streptomyces ahygroscopicus	1	0
Streptomyces aizunensis	1	1 (B-11277)
Streptomyces akiyoshiensis	2	0
Streptomyces aureus	1	0
Streptomyces bottropensis	1	0
Streptomyces canescens	1	0
Streptomyces caniferus	1	0
Streptomyces carneus	1	0
Streptomyces flavovirens	5	1 (B-2685*)
Streptomyces gelaticus	1	0
Streptomyces geysiriensis	1	0

included 46 bacteria, 11 yeasts and five actinomycetes. In comparison with the data in our previous lipase screen [3], it is interesting to find that only 17 out of these 62 esterase-positive strains also produce the triacylglyceride hydrolysis activity, i.e., lipase activity. These 17 strains were indicated with an asterisk in Table 1.

To determine yields of 2-ethyl-1-hexanol from the hydrolysis of 2-ethylhexyl butyrate by these positive strains, experiments were conducted as described in Materials and Methods. Results obtained with these organisms as well as with commercial enzymes are listed in Table 2. The yields ranged from less than 1 to 79.1% depending on the strains used. The best yields (79.1%) were from *Pseudomonas myxogenes* NRRL B-19. Commercially available lipases and esterases hydrolyzed 2-ethylhexyl butyrate at a low to moderate level (2.5 to 46.2%).

Hydrolysis products were identified by comparison of GC retention times with those of authentic samples and by co-chromatography. Under our GC conditions, 2-ethyl-1-hexanol had a retention time of 5.5 min. Further confirmation was done with GC/MS. Molecular-ion peak and fragments observed from product were: *m/e* 112 (M-18), 98, 83, 70, 57 and 55 (Fig. 2) which were identical to those obtained from authentic 2-ethyl-1-hexanol.

Stereospecificity of the hydrolysis

The products (2-ethyl-1-hexanol and butyric acid) from the hydrolysis of 2-ethylhexyl butyrate by *Pseudomonas mucidolens* NRRL B-18 were subjected to optical rotation measurements. The specific optical rotation value of the crude product 2-ethyl-1-hexanol was $[\alpha]_D^{22} = +2.59$ (chloroform). The reported data [2] for the acid analogues of this compound are:

(S)-2-ethylhexanoic acid $[\alpha]_D^{22} = +7.2$ (chloroform)

(R)-2-ethylhexanoic acid $[\alpha]_D^{22} = -7.4$ (chloroform)

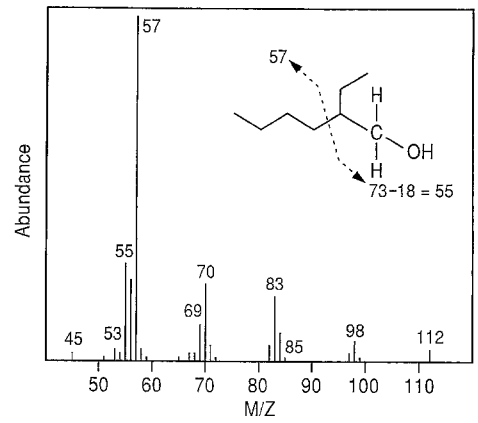

Fig. 2. EI mass spectrum of 2-ethyl-1-hexanol produced from microbial hydrolysis of 2-ethylhexyl butyrate.

TABLE 2

Production of 2-ethyl-1-hexanol by microbial asymmetric hydrolysis of 2-ethylhexyl butyrate

Microorganisms		Yield (%)	Stereospecificity[a]
Altermonas sp. NRRL B-808		40.2	rac
Bacillus amyloliquefaciens	B-545	26.8	S(+) 86%
Bacillus amyloliquefaciens	B-1466	18.6	nd
Bacillus megaterium	B-350	17.4	rac
Bacillus megaterium	B-1827	12.9	nd
Bacillus polymyxa	B-355	0.9	S(+) 62%
Bacillus subtilis	B-364	0.6	nd
Enterobacter aerogens	B-167	40.2	S(+) 81.6%
Flavobacterium aurantiacum	B-184	12.9	nd
Pseudomonas acidovorans	B-936	13.9	nd
Pseudomonas aeruginosa	B-23*	27	nd
Pseudomonas aeruginosa	B-257*	20	nd
Pseudomonas aeruginosa	B-275*	12	nd
Pseudomonas aeruginosa	B-2785	52.3	R(−) 68.4%
Pseudomonas aminovorans	B-934	44.2	rac
Pseudomonas antimycetica	B-1683*	45.6	S(+) 90%
Pseudomonas aromatica	B-2173	65.7	S(+) 84.4%
Pseudomonas berberidis	B-831	15.6	nd
Pseudomonas fluorescens	B-1609	36.2	S(+) 81%
Pseudomonas fluorescens	B-1612*	3.3	nd
Pseudomonas fluorescens	B-1636*	29.7	nd
Pseudomonas fragi	B-2316*	0.6	nd
Pseudomonas indigofera	B-2646	37.6	S(+) 65%
Pseudomonas maltophila	B-2337	32	S(+) 60%
Pseudomonas marginata	B-792*	23.1	nd
Pseudomonas mucidolens	B-16	29.5	S(+) 77.4%
Pseudomonas mucidolens	B-18*	45.6	S(+) 90%
Pseudomonas myxogenes	B-19	79.1	rac
Pseudomonas nonliquefaciens	B-994	46.9	S(+) 79.4%
Pseudomonas ribicola	B-151	30	rac
Pseudomonas ribis	B-160	33.6	nd
Pseudomonas echinoides	B-3127	11	nd
Pseudomonas seminum	B-2742	49.6	S(+) 70%
Pseudomonas septica	B-1963*	32	rac
Pseudomonas suis	B-823	0.3	nd
Pseudomonas suis	B-919	10.2	nd
Pseudomonas syncyanea	B-1246	9.3	nd
Pseudomonas viridilivida	B-721	11.3	nd
Pseudomonas viridilivida	B-1032	4.2	nd
Unknown isolates	B-1883	0.12	nd
Unknown isolate	ADM4	0.45	rac
Candida diddensiae	Y-6970	10.8	S(+) 69%
Candida fennica	Y-7505	3.8	nd
Candida humicola	Y-17222	1.3	nd
Candida ingens	Y-7796	9.1	nd
Candida maritima	Y-7899	0.46	nd
Pichia castillae	Y-7501	3.8	nd
Pichia farinosa	Y-2060	3.2	nd
Pichia inositovora	Y-12698	17.9	rac
Unidentified isolate	B-2877	0.91	nd
Zygosaccharomyces bisporus	Y-12626	2.7	nd

TABLE 2

(continued)

Microorganisms		Yield (%)	Stereospecificity[a]
Chainia purpurogenia	B-2952	23.7	nd
Chainia violens	B-3483	41.4	nd
Streptomyces aizunensis	B-11277	0.3	nd
Streptomyces flavovirens	B-2685*	59.5	rac
Commercially available lipases from			
Candida cylindracea (Type VII)		18.4	rac
Porcine pancreas (Type II)		46.2	S(+) 58.7%
Pseudomonas spp. (Type VIII)		19.0	S(+) 64.5%
Rhizopus arrhizus (Type XI)		28.8	S(+) 53.9%
Wheat germ (Type 1A)		2.5	rac
Commercially available esterases from			
Porcine liver		5.7	rac
Rabbit liver		3.0	rac

* Also lipase screen positive [3].

[a] rac = racemic; nd = not done; % = GC percent of major enantiomer.

Because the acid and the alcohol forms do not involve changes of the chiral center at C-2, both 2-ethylhexanol and 2-ethylhexanoic acid should possess identical optical properties. Therefore, the microbial hydrolysis product, is likely to be the S(+) isomer. The stereospecificity of the products from microbial hydrolysis of 2-ethylhexyl butyrate was also determined by GC analyses with a β-cyclodextran chiral column. Results obtained by this analyses are expressed as GC area percent of major enantiomer and listed in Table 2. Among the products analyzed, nine strains produced racemic mixtures, 13 strains produced preferentially the S(+) isomer and one strain produced preferentially the R(−) isomer of 2-ethyl-1-hexanol. The best strains for the enantioselective hydrolysis to yield the S(+) isomer are *Pseudomonas mucidolens* NRRL B-18 and *Ps. antimycetica* NRRL 1683 with 90% or 80% enantiomeric excess (e.e.). The only strain hydrolyzing the R(−) isomer of the substrate is *Pseudomonas aeruginosa* NRRL B-2785 with 68.4%. Commercially available lipases and esterases showed no enantiospecificity in the hydrolysis of 2-ethylhexyl butyrate (Table 2).

There has been long-standing interest in the asymmetric synthesis of organic compounds and in the asymmetric resolution of racemic esters. Recently, several examples of enzymic approaches have been reported [1,7,9]. Commercially available lipases have been used in these types of studies. Although some lipases can function as an esterase, they are not as good as esterase in catalyzing the reactions on organic esters. In comparing our lipase screening data [3] with our findings here (Table 1), it is clear that not all lipases are active toward 2-ethylhexyl butyrate. The data present here provide important information for the discovery of new stereospecific esterases.

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