Pseudomonas sp. Lipase-Catalyzed Synthesis of Geranyl Esters by Transesterification

Lisa N. Yee[a], Casimir C. Akoh[a,*] and Robert S. Phillips[b]

[a]Department of Food Science and Technology, Food Science Building, and [b]Departments of Chemistry and Biochemistry, The University of Georgia, Athens, Georgia 30602-7610

ABSTRACT: *Pseudomonas* sp. lipase was immobilized by adsorption onto five supports and tested for its ability to synthesize geranyl esters by transesterification using short-chain triacylglycerols as acyl donors. Reaction mixtures were prepared in 2 mL of *n*-hexane, 0.1 M geraniol, 0.03M triacylglycerol, and 200 units of lipase, and incubated at 30°C and 200 rpm for 24 h. Overall, glass beads were the best support. Geranyl acetate and caproate performed best with Duolite (77.5 and 95.3%, respectively). Geranyl butyrate and caprylate performed best with polyvinylpyrrolidone, (80.2 and 95.5%, respectively). Values for nonimmobilized enzyme also were obtained. Immobilization improved yields, with geranyl caproate exhibiting best results. *JAOCS 72*, 1407–1408 (1995).

KEY WORDS: Adsorption, geranyl esters, immobilization, lipase, *Pseudomonas sp.*, short-chain triacylglycerols, transesterification.

Terpene esters of short-chain fatty acids, especially those of geraniol, are important flavor and fragrance compounds widely used in the food and beverage and cosmetic and pharmaceutical industries (1,2). Products from enzyme-mediated reactions can be considered natural and may have high economic value (3). Even though chemical synthesis may be more economical in some cases, enzymic reactions require less energy and obtain higher quality products due to stereoselective and regioselective transformation of substrates (4,5). Lipases (glycerol ester hydrolase EC 3.1.1.3) can catalyze synthetic and group exchange reactions when water is replaced by an adequate organic solvent, such as *n*-hexane, thereby shifting the reaction equilibrium toward synthesis instead of hydrolysis (6). Lipases have been used as biocatalysts for the production of flavor esters by transesterification (7–10), and may show differing potentials for ester synthesis. Both immobilized and nonimmobilized lipases can be used in transesterification reactions. In this study, a *Pseudomonas* sp. lipase (PS) was immobilized by adsorption onto five different supports and tested for its ability to catalyze the synthesis of geranyl esters by transesterification of short-chain triacylglycerols. Results were compared to those obtained with nonimmobilized lipase.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials. Nonspecific lipase PS (33,000 U/g) from *Pseudomonas* sp. in powdered form was obtained from Amano International Enzyme Co. (Troy, VA). Geraniol (95% pure), triacetin, tributyrin, tricaproin, and tricaprylin (all 99% pure), and support matrix polyvinylpyrrolidone (PVP) were purchased from Sigma Chemical Co. (St. Louis, MO). *n*-Hexane (high-performance liquid chromatographic grade) and Celite 545 diatomaceous earth were from Fisher Scientific (Norcross, GA). Duolite A-340 ion-exchange resin and silica gel 12, 28–200 mesh size, were purchased from Aldrich Chemical Co. (Milwaukee, WI), and glass beads (3 mm) were obtained from Corning Incorporated (Corning, NY).

Experimental procedure. The crude lipase preparation was immobilized by simple adsorption technique onto five different support matrices. One gram of support was washed three times with 5 mL of distilled water and dried in an oven at 80°C. One gram of lipase PS was dissolved in 1 mL of distilled water. The dried support was added to the enzyme solution, mixed well with a stirring rod, and spread onto filter paper. This was then dried on a desiccator overnight at room temperature and stored in glass vials in the refrigerator until use.

Ester synthesis was carried out in duplicate with 200 units of lipase PS, as previously described by Yee *et al.* (10) with triacetin, tributyrin, tricaproin, and tricaprylin as acyl donors. Controls with no enzyme also were incubated. Reaction mixtures were prepared in 2 mL of *n*-hexane, 0.1 M geraniol, 0.03 M triacylglycerol, and 200 units of lipase, and incubated at 30°C and 200 rpm for 24 h. Samples were extracted and analyzed with a Hewlett-Packard HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector, as previously described (10). Synthesis of terpene esters was calculated from the amount of geraniol that reacted, and were further quantified by an online computer (9,10).

RESULTS AND DISCUSSION

The extent to which lipase PS, immobilized onto different supports, synthesized geranyl esters is shown in Table 1. Compared to the other supports, Celite and silica gel per-

TABLE 1
Yields of Geranyl Esters Catalyzed by *Pseudonomas* sp. (PS) Immobilized by Adsorption onto Different Matrices[a]

	Support matrix				
Geranyl ester	Celite	Duolite	Glass beads[b]	PVP	Silica gel
Geranyl acetate	72.1	77.5	72.4 (74.3)	71.0	61.8
Geranyl butyrate	54.6	77.3	78.2 (64.5)	80.2	62.9
Geranyl caproate	80.4	95.3	95.0 (80.0)	90.2	60.7
Geranyl caprylate	76.0	85.9	94.9 (93.2)	95.5	79.0

[a]Yields are expressed in percent yield. Reactions were performed in duplicate in 2 mL hexane with 0.1 M geraniol, 0.03 M triacylglycerol, and 200 units of lipase PS. PVP, poly(vinyl)pyrrolidone.
[b]Numbers in parentheses are nonimmobilized powdered lipase as control.

formed poorly, showing the lowest overall values for all triacylglycerols chainlengths used. Duolite, glass beads, and PVP gave acceptable yields of up to 95.5%. Duolite and glass beads gave best yields for geranyl acetate and caproate. For geranyl butyrate and caprylate, the best supports were PVP and glass beads. Glass beads seemed to be the best overall support matrix, followed by PVP and Duolite. With some exceptions, as the chainlength increased, yields increased, suggesting that lipase PS may have a higher affinity for fatty acid chainlengths greater than four. This supports our previous report, in which lipase from *Mucor miehei* showed increasing affinity for increasing fatty acid chainlength (1). In the present study, the influence of the support matrix cannot be discounted, though it is poorly understood at this time. Certainly this observation warrants further investigation. Yields obtained for nonimmobilized lipase PS also were compared to those obtained with enzyme immobilized onto glass beads. For the powdered form, yields increased with an increase in chainlength, except for geranyl butyrate. Yields were even better than those obtained when the enzyme was immobilized onto silica gel. In our previous study, in which powdered lipase PS was used to catalyze the synthesis of terpene esters by transesterification (8), values close to the ones reported here were obtained with triacylglycerols as the acyl donor. Thus, the present study confirms our earlier observations (8). Except for geranyl acetate, for which the yield was actually lower for immobilized lipase compared to powdered form, all other terpene esters showed an increase in yield due to immobilization onto glass beads. Significantly higher yields were obtained for geranyl butyrate and caproate with the immobilized enzyme compared to the yields obtained with the powdered enzyme. Overall, geranyl caproate gave the best results and improvement due to immobilization compared to other terpene esters. Gas-chromatographic retention times for linalool (internal standard), geraniol, and geranyl caproate were 3.6, 6.1, and 14.4 min, respectively. In this study it is evident that some supports did not work well for the *Pseudomonas* sp. lipase. Also, the acyl donor chainlength, as well as the immobilization method, may affect the yield. Thus, studies with lipase PS are of great interest because of its potential usefulness in flavor ester synthesis and other biotechnological applications.

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