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Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties

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Abstract

The lipases from *Thermomyces lanuginosus* (immobilized by granulation with silica) and C*andida antarctica* B (adsorbed on Lewatit, "Novozym 435"*)* were comparatively assayed for the synthesis of sugar esters by transesterification of sugars with fatty acid vinyl esters in 2-methyl-2-butanol:dimethylsulfoxide mixtures. We found that lipase from *C. antarctica* B is particularly useful for the preparation of 6,6′-di-acylsucrose, whereas *T. lanuginosus* lipase catalyzes selectively the synthesis of 6-*O*-acylsucrose. The granulated *T. lanuginosus* lipase retained more than 80% of its initial activity after 20 cycles of 6 h. Both lipases were similarly effective for the regioselective synthesis of 6′-*O*-palmitoylmaltose and 6-*O*-lauroylglucose. The effect of the synthesized sugar esters on the growth in liquid medium of various microorganisms (Gram-positive, Gram-negative and yeasts) was evaluated. 6-*O*-lauroylsucrose and 6′-*O*-lauroylmaltose inhibited the growth of *Bacillus* sp. at a concentration of 0.8 mg/ml, and of *Lactobacillus plantarum* at 4 mg/ml. Sucrose dilaurates and 6-*O*-lauroylglucose did not show antimicrobial activity, probably due to their low aqueous solubility. As regards the inhibition of yeasts, none of the tested carbohydrate esters inhibited significantly the growth of *Zygosaccharomyces rouxii* and *Pichia jadinii*.

Keywords: Carbohydrate esters; Sucrose esters; Maltose esters; Glucose esters; Lipases; Enzymatic transesterification; Enzyme granulation; Vinyl fatty acid esters; Antimicrobial activity

1. Introduction

Sugar fatty acid esters, synthesized from renewable resources such as fatty acids and carbohydrates, have broad applications in the food industry [1,2]. Other fields of application include cosmetics, detergents, oral-care products and medical supplies. Their antimicrobial [3], antitumoral [4] and insecticidal [5] properties have been reported and might open new markets. Sucrose esters and methyl glucoside esters, by far the most developed derivatives of this group, are being produced at about 4000 and 2000 Tm/year, respectively [6].

Regioselective acylation of carbohydrates is an arduous task due to their multifunctionality [7]. Sugar esters can be synthesized using either chemical or biological catalysts. Their current chemical synthesis is usually base-catalyzed at high temperatures, has a poor selectivity and gives rise to coloured side-products [8]. However, the enzyme-catalyzed processes are notably more selective [9]. For this purpose, lipases are the most useful enzymes. Furthermore, the two lipases most commonly used in sugar esters synthesis are those from *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*) and *Candida antarctica* B.

We recently developed a new and simple process for the lipase-catalyzed acylation of sucrose [10] and other di- and trisaccharides [11]. The method was based on the use of mixtures of a tertiary alcohol (2-methyl-2-butanol) and a polar

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solvent (dimethylsulfoxide) as reaction media, which represent a compromise between enzyme activity and sugar solubility. In this work, we have optimized the synthesis of various sugar mono- and diesters in solvent mixtures. We have compared the lipases from *T. lanuginosus* and *C. antarctica* B in terms of activity, regioselectivity and potentially reliable catalysts for sugar ester production.

An intensive effort is still being made in the screening of novel compounds able to inhibit or delay the growth of a range of microorganisms, for food and medical applications. In particular, sugar fatty acid esters display significant activity against several food and clinical isolates [3,12]. They have increasing interest due to advantages with regard to performance, consumers' health and environmental compatibility compared to petrol-derived standard products. By controlling the esterification degree, which may be modulated by the nature of the biocatalysts and the solvent composition, as well as the nature of fatty acid and sugar, it is possible to modify their properties. Taken this into consideration, the effect of the synthesized derivatives on the growth of a series of microorganisms involved in food spoilage was also investigated.

2. Experimental

2.1. Chemicals

Granulated lipase from *T. lanuginosus* and immobilized lipase from *C. antarctica* B (Novozym 435) were from Novozymes A/S. Maltose, glucose, molecular sieves (3 Å, 8–12 mesh) and 2-methyl-2-butanol (*tert*-amyl alcohol) were from Sigma. Sucrose and dimethyl sulfoxide (DMSO) were supplied by Merck. Vinyl laurate was from Fluka. Vinyl palmitate was from TCI (Tokyo, Japan). All other reagents and solvents were of the highest available purity and used as purchased.

2.2. Organisms

The microorganisms used in this study included *Bacillus* sp. CECT 40, *Pseudomonas fluorescens* CECT 378, *Staphylococcus aureus* CECT 240, *Erwinia carotovora* CECT 225T, *Escherichia coli* CECT 101, *Bacillus stearothermophilus* CECT 47, *Lactobacillus plantarum* CECT 220, *Zygosaccharomyces rouxii* CECT 1232 and *Pichia jadinii* CECT 1062. Microorganisms strains were maintained according the recommendations of the manual of the Spanish Type Culture Collection (CECT) (http://www.uv.es/cect/).

2.3. Enzymatic synthesis of sucrose esters

Sucrose laurate was synthesized by transesterification of sucrose (0.03 M) with vinyl laurate (0.3 M) in 2-methyl-2-butanol containing 20% of dimethylsulfoxide, in a 5 ml scale. Silica-granulated lipase from *T. lanuginosus* or the immobilized lipase from *C. antarctica* B (Novozym 435) were used as biocatalysts (100 mg/ml). Reactions were performed at 40 °C with orbital shaking (100 rpm) in the presence of 3 Å molecular sieves (100 mg/ml). Reactions were followed by HPLC, using a SP-8810 pump (Spectra-Physics Inc.), a Nucleosil 100-C18 column (250 mm × 4.6 mm, Análisis Vínicos, Spain), maintained at 40 °C, and a refraction-index detector model 2410 (Waters). The mobile phase was a 90:10 (v/v) methanol:water mixture at 1.1 ml/min. The products were isolated by column chromatography, and fully characterized by spectroscopic techniques (NMR, IR, HR-MS) as previously described [10].

2.4. Enzymatic synthesis of maltose esters

Maltose esters were synthesized by transesterification of maltose (0.03 M) with vinyl laurate or palmitate (0.15 M) in 2-methyl-2-butanol containing 20% of dimethylsulfoxide, in a 5 ml scale. The reaction mixture contained 25 mg/ml of biocatalyst and 25 mg/ml of 3 Å molecular sieves. Reactions were performed at 40 °C with orbital shaking (100 rpm). Reactions were followed by HPLC, using a 9012 pump (Varian) and a Nucleosil 100-C18 column (250 mm × 4.6 mm, Análisis Vínicos, Spain), maintained at 40 °C. Detection was performed using an evaporative light-scattering detector DDL-31 (Eurosep) equilibrated at 55 °C. Methanol:water 95:5 (v/v) containing 0.1% acetic acid was used as mobile phase (flow rate 1.2 ml/min) for 7 min. Then, a gradient from this phase to pure methanol was performed in 1 min at the same flow rate. Methanol was held as mobile phase at 1.2 ml/min during 12 min. The products were purified by solvent precipitation and column chromatography, and fully characterized by spectroscopic techniques (NMR, IR, HRMS), as previously described [11].

2.5. Enzymatic synthesis of glucose esters

Glucose laurate was synthesized by transesterification of glucose (0.3 M) with vinyl laurate (0.3 M) in 2-methyl-2-butanol. Reactions were performed at 40 °C with orbital shaking (100 rpm) in the presence of biocatalyst (100 mg/ml) and 3 Å molecular sieves (100 mg/ml). Reactions were followed by HPLC under conditions described above for sucrose esters, but using a mobile phase 85:15 (v/v) methanol:water. The products were isolated as previously described [13]. Briefly, the mixture was filtered, washed with 2-methyl-2-butanol, evaporated under vacuum, and the solid (white powder) was recrystallized in acetone and dried in vacuum. The products were characterized by spectroscopic techniques (NMR, IR, HRMS).

2.6. Analysis of antimicrobial properties in liquid medium

The analysis of antimicrobial activity of the carbohydrate esters was performed using different liquid media accord-