고정화된 *Candida antarctica*를 사용한 당 에스테르의 효소적 합성

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Enzymatic production of sugar fatty acid esters using the immobilized *Candida antarctica* lipase

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1. Introduction

Surfactants (surface active agents) are indispensable components of daily life. They widely used in the cosmetics, food industries, detergent industries and pharmaceutical applications. Many different types of surfactants are already being used in industry.

Sugar fatty acid esters (SFAE) are non-ionic surfactants and they have very good emulsifying, stabilizing, or conditioning effects and are derived from renewable feedstocks, inexpensive and readily available. The non-ionic surfactants occupy 24% in the surfactant markets. On the other hand, for a long time large scale production of sugar fatty acid esters remained mainly in the realms of organic chemistry and chemical processing. Chemical methods are mainly performed at high temperatures in the presence of alkaline catalysts. High energy consumption, coloring of products and low selectivity are major disadvantages of these methods. Moreover, some chemically synthesized sugar fatty acid esters are toxic and not readily biodegradable, thus causing their limited application in cosmetics, food industry and pharmaceutics where excellent toxicological properties are desired. In addition, the complete removal of toxic organic solvents such as DMSO and DMF used for the solubilization of sugar components is laborious [1-3].

Sugar fatty acid esters have been also produced by means of fermentation, resulting in the formation of isomeric mixtures of these so-called ‘biosurfactants’. They are commercially used in cosmetics and have been also explored in the field of tertiary oil recovery [4-5].

Biosurfactants – sugar fatty acid esters (SFEA) – have attracted the great attention of biotechnological researchers because they consist of two inexpensive, renewable and easily accessible starting agricultural materials – sugar and fat/oil [6-8]. In recent years, growing consumer demand for ‘green’ products has focused attention on the utilization of carbohydrates as raw materials for specialty

chemicals (Figure 1).

In recent years, the use of isolated enzymes as an alternative for the synthesis of sugar fatty acid esters has been explored, emphasizing the synthesis of regioselective pure products [9]. Esters synthesis in aqueous media has been reported, but with a low product yield [10]. In non-aqueous media, different strategies had been used to overcome solubility problems. Due to the low solubility of sugars in organic solvents, two different processes for the enzymatic synthesis of sugar fatty acid esters have been explored: (ⅰ) the use of bulky polar organic solvents such as pyridine or dimethylformamide or (ⅱ) attempts to enhance the reaction rate by the use of activated acyl donors, substrate immobilization or increase of substrate hydrophobicity to increase the miscibility of the substrates. Unfortunately the solvents used in the enzymatic synthesis of sugar fatty acid esters are often deleterious to most lipases, resulting in partial or complete deactivation. Furthermore, protecting and deprotecting steps may complicate product purification and increase process costs.

In this study, we investigated the sugar fatty acid esters in organic solvents through lipase catalyzed condensation of xylitol and fatty acids with carbon numbers of 16 to 18 in tert-butyl alcohol with low water content. Esterification activity was measured under various conditions.

2. Experimental

Novozym 435 (Immobilized lipase from *Candida actarctica*) was a gift from Novo-Nordisk (Bagsvaerd, Denmark). Xylitol, D-sorbitol, meso-erythritol, D(+)-glucose, sucrose were obtained from Sigma (USA). Acetone, acetonitrile, ethylacetate, tert-butyl alcohol, 2-Butanone, molecular sieve 4A 1/16 were purchased from Showa (Japan).

Reaction methods reported by Ljunger et al. have been modified [11]. Tert-butyl alcohol as 'adjuvant' was dehydrated over molecular sieve for 24 hours in 200℃ drying oven before use.

The solubilities of sugar were analyzed using HPLC (Agilent 1100 series, Refractive index detector) with Aminex HPX-87H column (300 * 7.8 mm, Biorad). The dehydrated tert-butyl alcohol was used as reaction medium for synthesis of sugar fatty acid esters. A typical condensation reaction of sugar ester was carried out as follows. A total of 6 mmol of sugars, 3 mmol of fatty acids and 10 ml of the dehydrated tert-butyl alcohol were put into glass vials with screw-capped. A total of the immobilized lipase (Novozym 435) corresponding to 0.25 wt% fatty acids was then added. The vials were screw-capped tightly and immersed into a thermo-regulated water bath at 60℃. The condensation reaction was carried out under reciprocal shaking at ca. 200 rpm. Under these conditions, some of the sugars added remained solid due to their low solubility. At appropriate intervals, a portion of the reaction mixture was sampled. The sugar fatty acid esters were analyzed using GC/Mass (HP-6890, Mass detector) with HP-1 capillary column (30 m*0.25 ㎜*0.25 ㎛ film thickness).

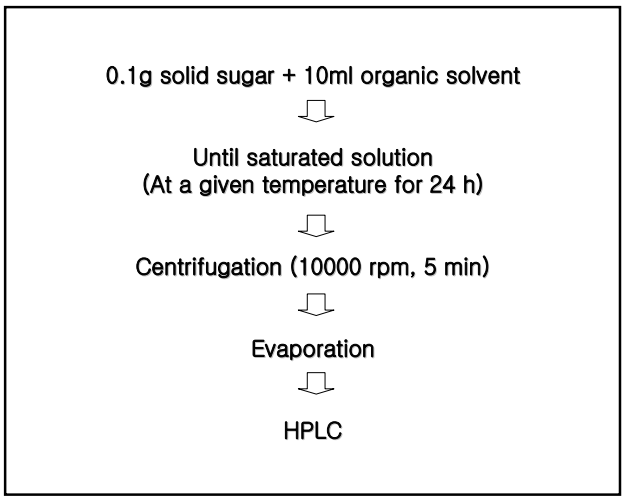

Fig.1. Determination of solubility of sugars

3. Results and discussion

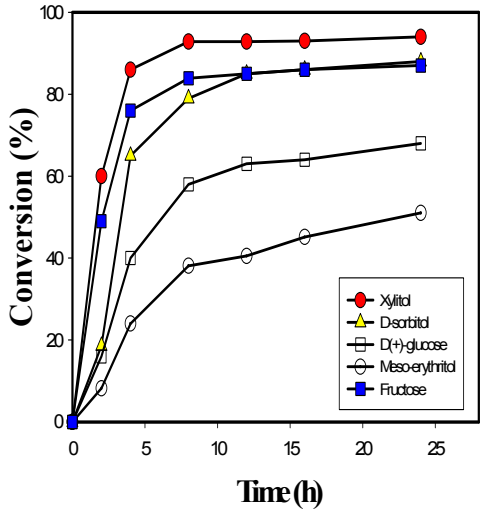

Fig.2. Time course of the synthesis of different sugar fatty acid esters in tert-butyl alcohol (sugar : oleic acid = 2:1)

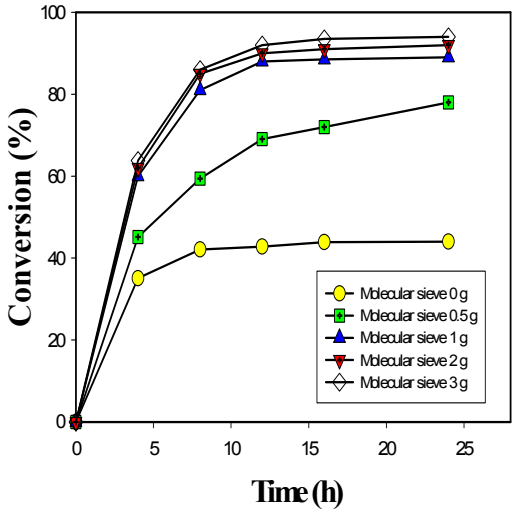

Fig.3. Influence of the molecular sieve addition (xylitol : oleic acid = 2:1)

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