

hydrolysis as a model reaction of double kinetic resolution (DoKR).

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27.

On the selectivity of *Mycobacterium* sp. NRRL B-3805 as steroid producer

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The production of steroid drugs and hormones is one of the best examples of the successful application of microbial technology in large-scale industrial processes. Given their properties, both biological and pharmacological, and the high cost associated to their synthesis, a great relevance is given to the efficient transformation of these compounds.

Concerning biotransformation, the selective cleavage of the side-chain of phytosterols by *Mycobacterium* sp. NRRL B-3805 cells, is a well-established multi-enzymatic process involving the use of nine catabolic enzymes in a 14-step metabolic pathway. The main product of this selective cleavage is 4-androstene-3,17-dione (AD), a key intermediate for the production of pharmaceutical steroids.

The present work aims to explore the biotransformation capabilities of *Mycobacterium* sp. NRRL B-3805 by studying the effect of structural variations in the steroid skeleton of the substrate in the catalytic activity. For this purpose, several steroids were used, natural and structurally modified, as well as pentacyclic terpenes, with different oxidation states on the A and B rings.

Different degrees of conversion were observed, depending on the structure of the substrate. Diosgenin and hecogenin, two steroids with a spiroketal side chain, remained unaltered. The pentacyclic triterpenoid, betuline, was also not accepted as substrate. On the other hand, several polyhydroxylated steroids were converted in AD.

These results show that, despite the different structural patterns of the steroids studied, the *Mycobacterium* sp. NRRL B-3805 is able to convert a variety of steroidal substrates into 4-androstene-3,17-dione.

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Production of polyglycerol polyricinoleate (PGPR) by using free and immobilized *Mucor javanicus* lipase

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Polyglycerol polyricinoleate (PGPR) is commonly used as emulsifier in the food industry. Known methods for preparing this compound involve the autocatalytic condensation of ricinoleic acid and alkali-catalysed reaction between the condensed ricinoleic acid and polyglycerol to give PGPR. These methods have the disadvantage of requiring very long reaction times and thus involving a large outlay and high-energy costs. The long reaction times also adversely affect the quality of the final product which presents problems of coloration and odours.

As an alternative, our research group is developing the enzymatic synthesis of PGPR by the catalytic action of lipase (E.C.3.1.1.3), which acts in mild reaction conditions of temperature and pressure and neutral pH. The enzymatic procedure consists of two steps. First the ricinoleic acid is polymerised by the action of *Candida rugosa* lipase to obtain the estolide. This process has been optimised by the authors and their investigations have given place to several publications (Bódalo et al., 2005; Bódalo et al., 2006a,b). Then the estolide is esterified with polyglycerol to yield PGPR. The second step is being optimized at present. Among 24 different lipases, three of them have been pre-chosen (Bódalo et al., 2006c) as catalyst of this reaction step and *Mucor javanicus* lipase is one of them.

Mucor javanicus lipase is a solid commercial product acquired from FLUKA, with specific activity 10 U/mg and protein content 23.5%. Esterification reactions have been carried out in a 250 ml open-air reactor at 40 °C, and the mass transfer was promoted by a quite powerful impeller stirrer which was used as mixing device. 33.75 g of polyricinoleic acid and 2.25 g of polyglycerol-3 (Solvay) were used as substrates and reaction course was monitored by the acid value (AV) measurement.

Enzyme activity has been tested by using three different operation protocols. First, it has been used as purchased and added to the reactor at $t=0$ (Fig. 1, blue series). Then the same enzyme amount was added as portions (100 mg/day) during the reaction process and a lower AV was reached (Fig. 1, green series). Finally, *M. javanicus* lipase was absorbed in an anion exchange resin (Lewatit MonoPlus MP64) (Bódalo et al., 2006a) and used in the same experimental conditions (Fig. 1, red series). The three alternatives require the same enzyme consumption and the best esterificación results are reached by using the immobilized