INFLUENCE OF DIFFERENT CARRIERS ON PHYTOSTEROL BIOTRANSFORMATION BY *MYCOBACTERIUM* SP.

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**Abstract:**
Androst-4-ene-3,17-dione (AD) and androsta-1,4-diene-3,17-dione (ADD) are important intermediates in the synthesis of steroidal medicines. They are obtained from side-chain cleavage of phytosterols by bacteria, mainly *Mycobacterium* species. The major obstacle in this process is the strong hydrophobicity of phytosterols leading to a low solubility in the aqueous media used to culture the bacteria. Many efforts have been carried out to improve the interaction between the phytosterol and the microorganism by using solubilizing agents. The aim of this work was to study different solubilizing agents in order to increase the bioavailability of phytosterols in the production of AD and ADD using *Mycobacterium*. Seven carriers, i.e., soybean oil, isopropyl alcohol, β-cyclodextrin, PEG 4000, PEG 400, PPG 612 and PPG 614, were studied. *Mycobacterium* sp. DSM 2966 was grown for 24 hours in 10 mL of medium (peptone, 5.0; meat extract, 3.0; yeast extract, 3.0; FeSO₄·7H₂O, 0.01; ZnSO₄·7H₂O, 0.02; MgSO₄·7H₂O, 0.05; dextrose, 1.0 per liter and pH 7.0) in 125 mL Erlenmeyer flasks under 180 rpm and 30 °C. This cell suspension was used to inoculate the biotransformation medium (peptone, 5.0; meat extract, 3.0; yeast extract, 3.0; FeSO₄·7H₂O, 0.01; ZnSO₄·7H₂O, 0.02; MgSO₄·7H₂O, 0.05 per liter and pH 7.0). The medium was supplemented with 1 (g.L⁻¹) phytosterol solubilized in the carrier under study. The reaction was performed in culture tubes containing 2 mL of culture medium. The incubation was done at the same conditions used to grow the inoculum. Samples were taken at 72, 120 and 168 h of cultivation and the products were extracted with ethyl acetate and analyzed by chromatography. The results indicated that isopropyl alcohol, soybean oil, PPG 614, β-cyclodextrin and PEG 4000 facilitated dissolution and bioavailability of phytosterol significantly. The best carrier for AD and ADD production was soybean oil in a biphasic system (phase ratio of 1:16, oil to aqueous). Soybean oil is abundant and cheap in Brazil therefore has great potential of application as a raw material in this process. No products (AD and/or ADD) were detected when PEG 400, PPG 612 or none of the solubilizing agents were used in the biotransformation medium.

**Keywords:** Androst-4-ene-3,17-dione, Androsta-1,4-diene-3,17-dione, Phytosterol, *Mycobacterium*.

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