COMPARISON OF THE ACTIVITY OF TRANSESTERIFICATION OF LIPASES THROUGH THE para-NITROFENIL PALMITATO ASSAY OF TWO GRAM (-) BACILLUS ISOLATED OF SOILS.

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Abstract

The goal of this work was to evaluate the lipases activity of two previously isolated bacterias gram(-) with the assay of *para*-Nitrophenyl Palmitate (*p*NPP). The experiments were carried out with dried whole-cells and lyophilized supernatant from two strains grown in two different media. The strains CN-123 and MMS-3 were cultivated in nutrient broth (CN) and a mineral media added with sucrose, respectively. Both media were adjusted at pH 7 and 5% (w/w) of waste cooking oil and incubated at 30°C for 32 h. The media cultures were centrifuged and the supernatants obtained were lyophilized (SL) and the cell pellets were air dried (CS). Samples of 20 mg of each SL and CS were taken for reaction procedure and samples were taken at reaction at times of: 0.5, 5, 10, 20, 40 and 60 minutes. In case of whole cells, the quantity of *para*-Nitrophenol (pNP) was apparently constant during along the experiment for both strains and both media. However, most of the activity of lipase (releasing of *p*NP) occurred at the beginning of the experiment. The maximal activity was quantifying in the strain MMS-3. In case of SL, the lipase activity was minor compared to that determined in whole cells. Our results demonstrated that the liberation of pNP occurred during the beginning of the experiments. Hence the lipase activity is high during the first times of reaction. These results demonstrated the effectiveness of pNPP assay for quantification and comparison lipase activities in the screening of different enzymes with novel characteristics.

Resumen

El objetivo de este trabajo fue el de evaluar la actividad de lipasas de dos bacterias Gram(-) aisladas de suelos previamente con el ensayo de *para*-Nitrofenil Palmitato (pNPP). Los experimentos se llevaron a cabo con células secas y sobrenadante liofilizado de dos cepas crecidas en dos diferentes medios. Las cepas CN-123 and MMS-3 fueron cultivadas en caldo nutritivo (CN) y en medio mineral adicionado con sacarosa, respectivamente. Ambos medios fueron ajustados a pH 7 y 5% de aceite de desecho de restaurantes y se incubaron a 30ºC por 32 h. Los medios de cultivo fueron centrifugados y los sobrenadantes obtenidos fueron liofilizados (SL) y los paquetes celulares fueron secados (CS). Muestras de 20 mg de cada SL y CS fueron tomados para llevar a cabo la reacción y muestras fueron tomadas a diferentes tiempos reacción de: 0.5, 5, 10, 20, 40 y 60 minutos. En el caso de las células completas, la cantidad de *para*-Nitrofenol (pNP) fue aparentemente constante durante todo el experimento para ambas cepas y ambos medios. Sin embargo, la mayoría de actividad de lipasa (liberación de pNP) ocurrió al principio del experimento. La actividad máxima fue cuantificada en la cepa MMS-3. En caso de SL, la actividad de la lipasa fue menor comparada a la determinada en la célula. Nuestros resultados demuestran que la liberación del pNP ocurre durante el inicio de los experimentos. Por lo tanto la actividad de lipasa es alta durante los primeros momentos de la reacción. Estos resultados demuestran la efectividad del ensayo de pNPP para la cuantificación y comparación de actividades de lipasas en la selección de diferentes enzimas con características novedosas.

Introduction

Biodiesel has gained importance in the recent past for its ability to replace fossil fuels which are likely to run out within a century. The environmental issues concerned with the exhaust gases emission by the usage of fossil fuels also encourage the usage of biodiesel which has proved to be eco-friendly far more than fossil fuels [1]. In general, biodiesel is often associated with methyl esters produced by the alkali-catalyzed transesterification of refined vegetable oils and fats with methanol. The production of biodiesel from unrefined feedstocks and greases using this technology, however, has encountered some difficulties as a result of the properties of these feedstocks and/or their free fatty acid content. Because of this, there is interest in the development of alternative methods, particularly biocatalytic ones, for producing biodiesel from these feedstocks. Several studies have reported the enzymatic alcoholysis of vegetable oils and animal fats in solvent and solvent-free systems, using primary and secondary alcohols.

The lipases (triacylglycerol acylhydrose; EC 3.1.1.3) are biocatalyst with significant industry application due to their diversity. Currently, the increase of new applications of lipases such as biosensors, chemicals, pharmaceuticals, pesticides, food, leather, detergents and cosmetics, many enzymatic activity assay methods are developed every year. Some of the reactions of industrial interest are the esterificación and transesterification in which the utilization of intracellular lipases in the form of whole-cell biocatalysts is both more cost-effective and more advantageous (2). Actually the use of lipases in process like the production of biodiesel is limited for their cost.

There are some methods to identify the activity of lipase (Table 1) .A fast and cheap test to measure the synthetic activity of the lipases is the spectrophotometric assay that consisted in the transesterification between the *para*-nitrophenil palmitate (*p*NPP) and methanol in an organic solvent. The method is based on lipase-catalyzed transesterification reactions between *p*NPP esters and methanol. In this reaction is released *para*-nitrophenol (*p*NP) that is detected in a common spectrophotometer at 410 nm.

Objective

The goal of this work was to evaluate the activity of lipases through *p*NPP assay in whole dry cells as well as in lyophilized supernatant of two bacterias gram (-). (4)