IMAGE PROCESSING TOOL FOR THE DETECTION AND QUANTIFICATION OF XYLANASE ACTIVITY IN A METAGENOMIC STUDY

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PO - (461) - IMAGE PROCESSING TOOL FOR THE DETECTION AND QUANTIFICATION OF XYLANASE ACTIVITY IN A METAGENOMIC STUDY

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Body

The vast diversity of unexplored microbial communities inhabiting the planet drives the continuous screening for promising biocatalysts. Until recently, the strategies to find new microorganisms and their enzymes were mainly focused on laboratory studies of pure microbial cultures. However, a great amount of environmental microorganisms cannot be cultivated under laboratorial conditions [1]. Metagenomics has emerged as an innovative approach to explore these uncultivable microorganisms through the analysis of DNA extracted from environmental samples [2]. It is considered a powerful tool for the discovery of novel biocatalysts and two different approaches have been proposed. Sequence-based studies recognize candidate genes but do not provide direct conclusions about the functionality of the encoded enzymes. On the other hand, the function-based approach allows the identification of new enzymes and also leads to preliminary information about their activities and physicochemical parameters. Indeed, function-based screenings have been successfully used in different environments to find genes encoding lignocellulose-degrading enzymes, such as xylanases [2]. These enzymes are considered important catalysts in the biological decomposition of lignocellulosic residues.

In this study, a fosmid library previously prepared in *Escherichia coli* with genomic DNA extracted from a compost sample collected in a national composting unit (Lipor) [3] was evaluated through a functional screening. To assess the xylanase activity of all the clones, a fast and simple chromogenic screening test using AZCL-xylan was performed in 96-well microplates at room temperature. Afterwards, the positive clones were selected and incubated at different temperatures (25, 37, 45 and 60 ºC) with the same substrate in Petri plates, for three days, to identify the most fast and promising clones. The presence of blue color was assumed as positive responses correlated with area's size. Area boundaries were extracted automatically by analyzing color images of the samples using MATLAB's in-house functions. At 60 ºC, no positive clones were detected. Two positive clones simultaneously exhibited enzymatic activity under 25, 37 and 45 ºC. In general, 37 ºC proved to be the most suitable temperature for the detection of xylanase activity. The method herein reported can be further optimized for the automatic detection of different enzymatic activities in high throughput screenings.

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