175. Functional screening for the detection of β-glucosidase activity in a metagenomic library obtained from a compost sample

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There is an increasing need to find novel and robust biocatalysts with promising features that compete with those currently available on the market. Composting is an extreme habitat of high microbiological diversity that represents a suitable source of lignocellulose-degrading enzymes, such as cellulases, hemicellulases and ligninases, properly active under harsh conditions. These enzymes can convert the recalcitrant structure of lignocellulose into valuable bioproducts with great biotechnological potential. β-Glucosidases are glycoside hydrolases responsible for degrading cellulose, namely in the disruption of the final glycosidic bonds of short-chain oligosaccharides to obtain glucose. Metagenomics is an emerging culture-independent technique that has proven effective in identifying new biocatalysts with better catalytic activity through the analysis of DNA extracted from a vast number of environments. The metagenomic analysis is divided into two main technologies: sequence- and functional-based approach. Function-based screening aims to discover and identify new genes capable of producing biocompounds/biomolecules with new or improved functions. This screening is based on the detection and isolation of clones with a positive response to the desired phenotype when activity-based techniques are applied. In this study, high-molecular-weight DNA extracted from a compost sample was used to construct a fosmid metagenomic library. This library was evaluated through a functional screening to identify clones that expressed cellulase activity, specifically β-glucosidase activity. The enzymatic activity was unravelled using esculin as substrate through the formation of a brown halo as a positive response (**Figure 1**). The functional screening was performed in 96-well microplates and the detection of β-glucosidase activity was evaluated at different temperature (25-60 °C) and pH (4.5-9.5) conditions. It was possible to identify clones with the enzymatic activity of β-glucosidase in almost all tested conditions, except at 60 °C. The best conditions for clone growth occur in a longer initial incubation time (3 days, 37 °C). On the other hand, the lower pH and incubation temperature favoured a faster detection of β-glucosidase activity.

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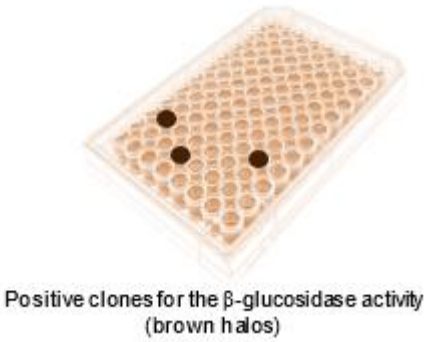

Figure 1. Functional screening performed in 96-well microplates for the β-glucosidase activity in the presence of esculin as substrate. The colour change of the agar culture medium to brown colour is indicative of a positive result.