BIOCATALYSIS OPEN DAY 2020
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Functional screening of metagenomics libraries to find cellulolytic enzymes

Joana Sousa, Ângela M. A. Costa, Andréia O. Santos, Eduardo J. Gudiña, Joana L. Rodrigues, Sara S. Silvério, Lígia R. Rodrigues

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Abstract

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 in laboratorial conditions.

Metagenomics has emerged as an innovative and strategic approach to explore these uncultivable microorganisms through the analysis of DNA extracted from environmental samples. Two different approaches have been proposed for metagenome research, namely function- and sequence-based technologies. Sequence-based studies identify 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 identify genes encoding lignocellulose-degrading enzymes, such as cellulases, xilanases or laccases. These enzymes are considered important catalysts in the biological decomposition of lignocellulosic residues.

In this study, a fosmid library previously prepared with genomic DNA extracted from composting residues was evaluated through a functional screening. To assess the cellulolytic activities of the *Escherichia coli* clones, fast and simple screening tests were used and the results obtained were carefully compared. The screening tests were performed in agar plates with the addition of suitable chromogenic or non-chromogenic substrates to the culture media. For the non-chromogenic substrate, appropriate dyes were used for staining and detection of potential enzymatic activity.

In this comparative study, it was concluded that some substrates and methodologies are more suitable and practical for the identification of cellulolytic positive clones.

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Introduction

Lignocellulosic residues – composting:

Great potential to be used as low-cost and bio-renewable

- ✓*Largely available around the world*
- ✓*Composed of cellulose, hemicellulose and lignin*
- ✓*Promising source of new thermophilic microorganism* specialized in the degradation of lignocellulosic materials and further conversion to added-value products - ***enzymes***
- ✓More than 99% of microorganisms from natural environments cannot be efficiently cultivated

METAGENOMICS - innovative and strategic approach

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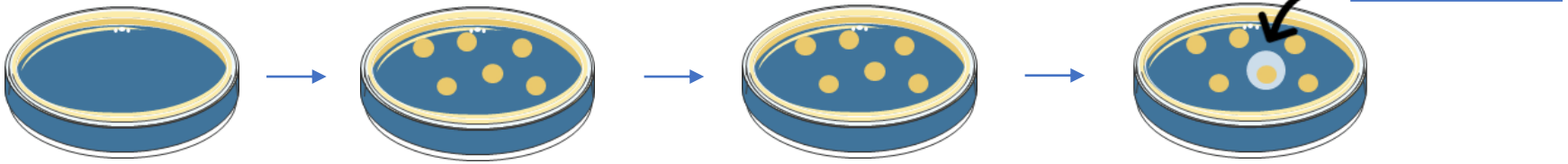
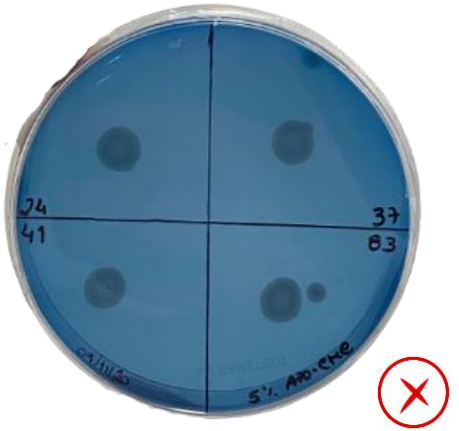
Experimental

Results

AZO-CMC (*endoglucanase*):





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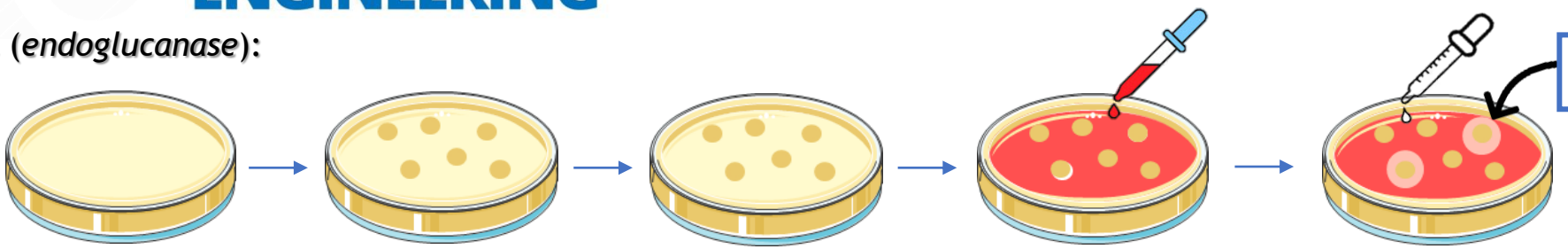
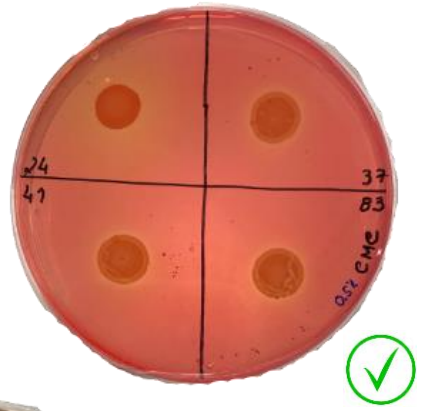
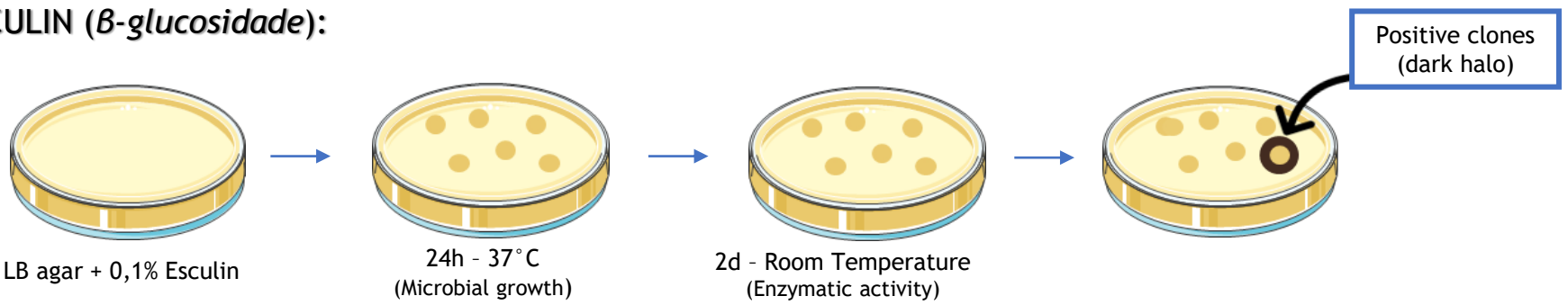
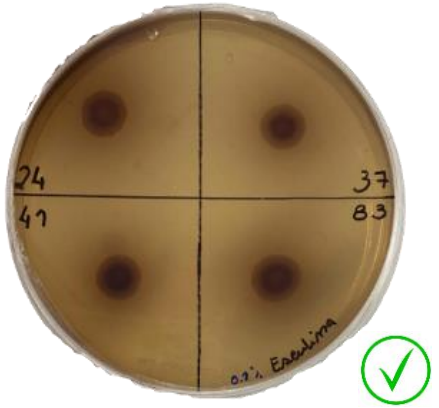
CMC (*endoglucanase*):





ESCULIN (*β-glucosidade*):





Conclusions

- ✓ Esculin and CMC proved to be the most suitable substrates to detect cellulolytic clones (ß-glucosidase and/or endocellulases activity). For AZO-CMC, no positive response was observed;
- ✓ The positive clones were firstly visualized using esculin (<48hours);
- ✓ Although the positive results obtained using CMC, this methodology was considered more tedious since additional steps were needed.

The study received financial support from Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and the project LIGNOZYMES (POCI-01-0145-FEDER-029773). The authors also acknowledge BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund (ERDF) under the scope of Norte2020 – North Portugal Regional Program. Joana Sousa also acknowledges her research grant (UMINHO/BIM/2020/28) from FCT.