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Tailor-made biocatalysts from environmental samples discovered through functional metaproteomics

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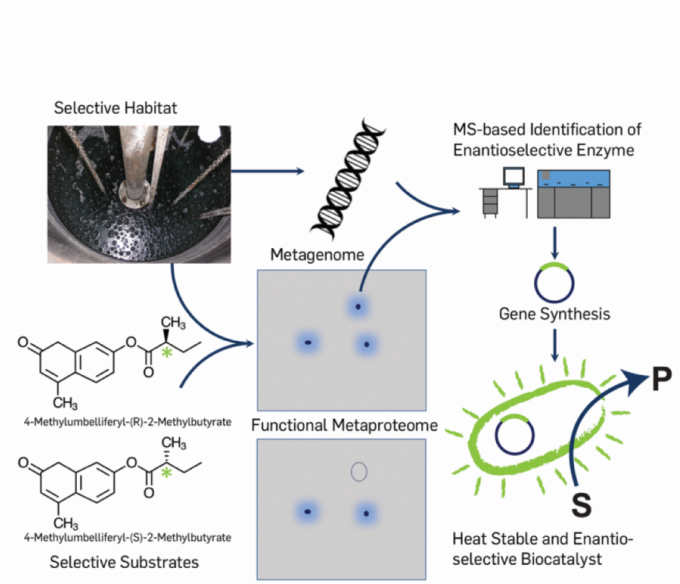
PURPOSE OF THE ABSTRACT
Bacterial biocatalysts play a key role in our transition to a bio-based, post-petroleum economy. Currently there is a limited number of commercially available biocatalysts, which often need to be laboriously adapted to a specific use case by enzyme and/or process engineering. However, given the diversity of microbial ecology, it is conceivable that natural evolution has already created a suitable biocatalyst that merely needs to be discovered.

Here, we present a simple workflow that combines functional metaproteomics and metagenomics, which facilitates the unmediated and direct discovery of tailor-made biocatalysts from environmental samples. To identify the entirety of biocatalysts with a desired activity in a soil sample (here: esterases/lipases), we detected all proteins active against fluorogenic substrates in the sample's metaproteome using a 2D-gel zymogram. The enzymes' primary structures could then be deduced by tryptic in-gel digest and mass spectrometry of the active protein spots, searching against a metagenome database created from the same contaminated soil sample [1]. These novel biocatalysts could then be obtained in quantity through heterologous expression of a synthetic gene in Escherichia coli for further characterization [2]. Using chain-length specific and enantioselective substrates, we can directly determine biocatalysts with desirable traits in environmental samples.

FIGURES



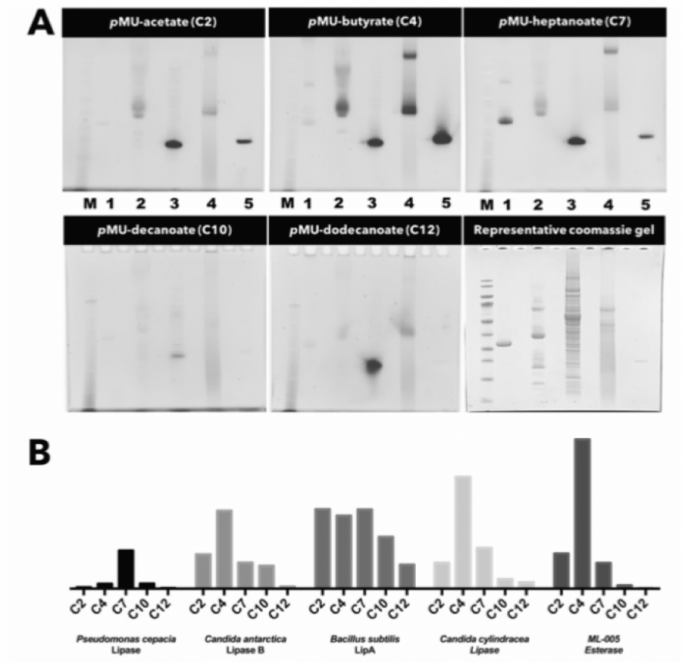


FIGURE 1

Biocatalyst discovery through metaproteomics

Schematic overview of a zymography-based, metaproteomic biocatalyst discovery workflow. The metaproteome of a promising bacterial community is separated on a 2D gel. Enantioselective fluorogenic substrates and MS are used to identify biocatalysts.

FIGURE 2

In-gel substrate specificity assay

In-gel determination of substrate specificity of a panel of selected commercial and metaproteomic lipases/esterases. (A) Zymograms using fluorogenic substrates with varying chain-lengths. (B) Relative activity of selected biocatalysts.

KEYWORDS

Biocatalysts | Lipase | Metaproteomics | Metagenomics

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