Paper Presentation

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Real-Time Screening of Biocatalysts in Live Bacterial Colonies

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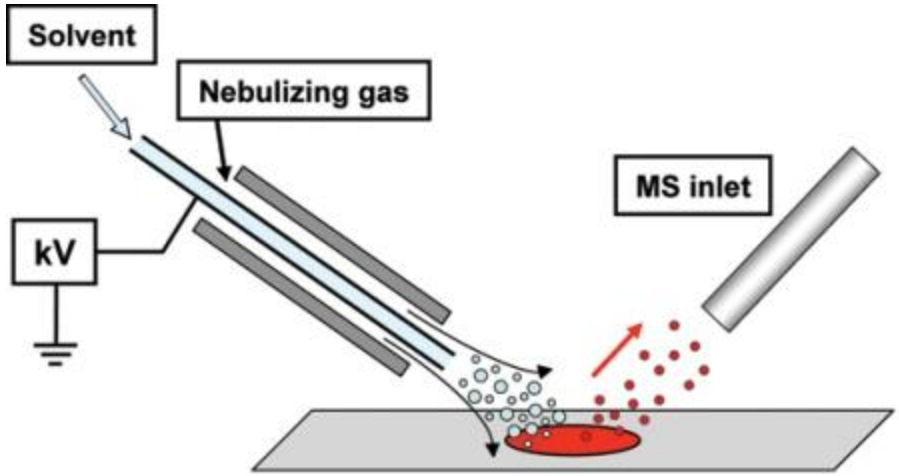
Introduction

➢ Screening of bacterial colonies to identify new biocatalytic activities is a widely adopted tool in biotechnology, but is constrained by the requirements for colorimetric or tag-based detection methods.



➢Mass spectrometry (MS) with its unique combination of specificity, sensitivity, and speed is an obvious analytical route for screening biotransformation reactions.

➢Desorption electrospray ionization allows one to look at this biotransformation in situ and in ambient condition with no or less sample preparation.

In this paper.....

➢Here they report a label-free on-colony screening method based on ambient ionization coupled with imaging MS, integrating the following three components provided by off-the-shelf instrumentation:

(i) DESI to ionize substrates and reaction products from the biotransformation;

(ii) ion mobility (IM) spectrometry to separation the plethora of ions produced following DESI from live colonies, which dramatically reduces background chemical noise;

(iii) imaging MS to locate the colony on the plate from distinctive lipid signals, and to identify specific product species from successful biotransformations.

➢They have term this on colony method Direct BioTransformation IMMS (DiBT-IMMS).

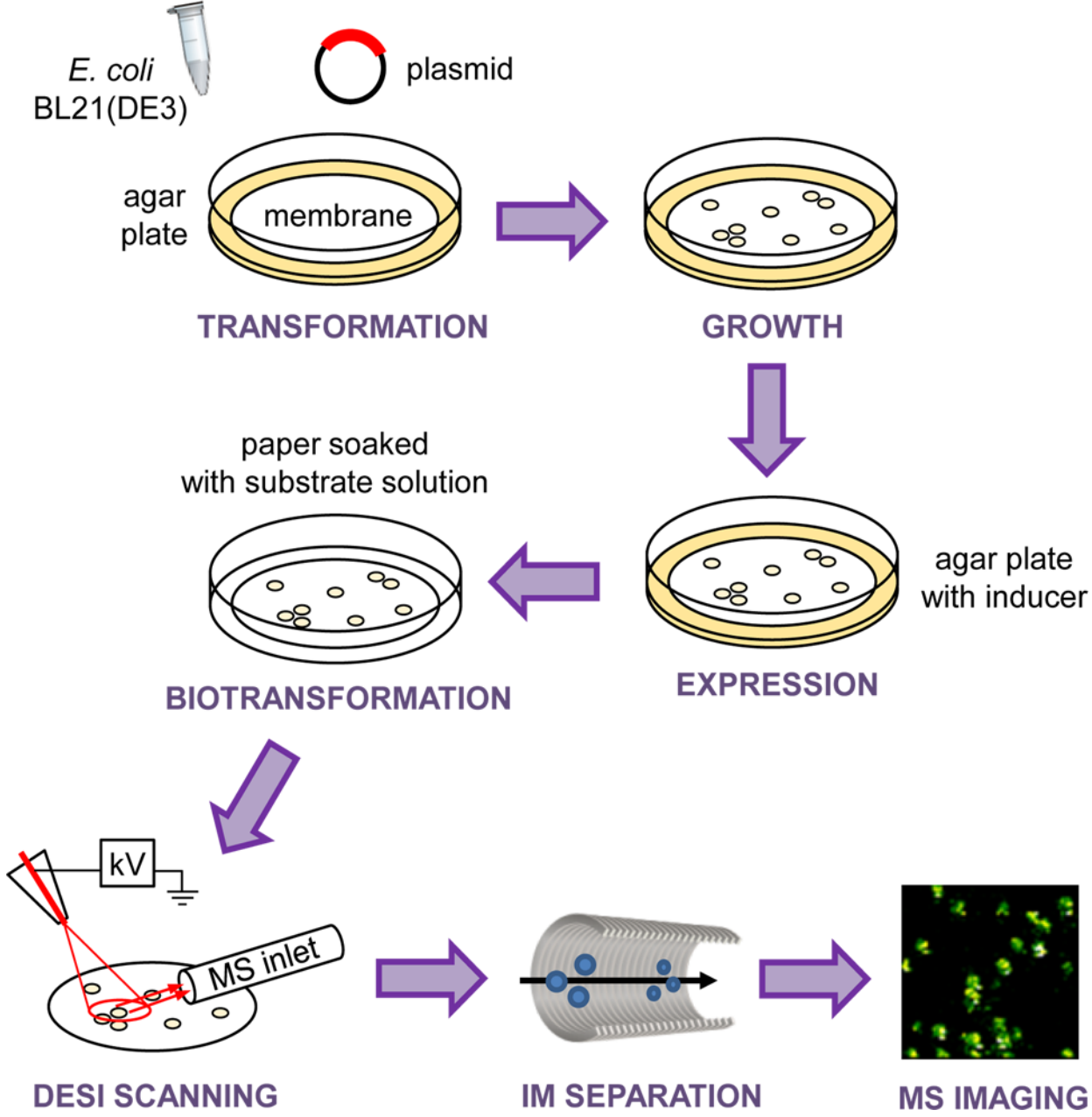


Figure 1. Workflow of DiBT-IMMS imaging of bacterial colonies expressing biocatalysts on agar plates under ambient conditions.

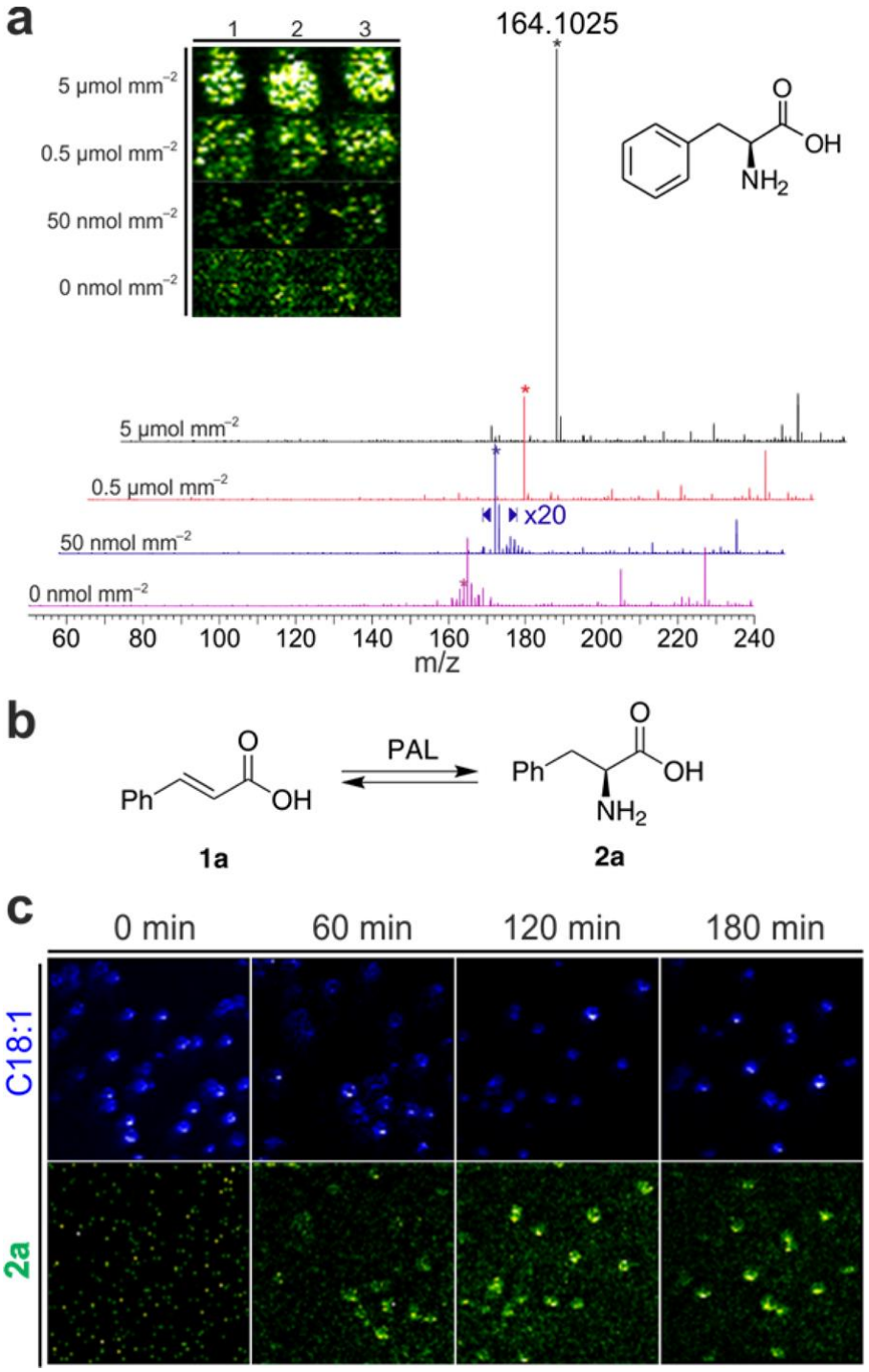


Figure 2. DiBT-IMMS screening of bacterial colonies. (a) Mass spectra illustrating the sensitivity of this technique employing Lphenylalanine as a model target compound (asterisk labeled peak). (b) Model PAL reaction examined here. (c) DiBT-IMMS mass-selected images from colonies expressing PAL, during the incubation with 1a (blue: selection of ion m/z 281 assigned to the cell wall lipid C18:1; green: selection of ion m/z 164 assigned to L-Phe 2a). The four samples were cut from the same membrane and incubated with the substrate for different times.

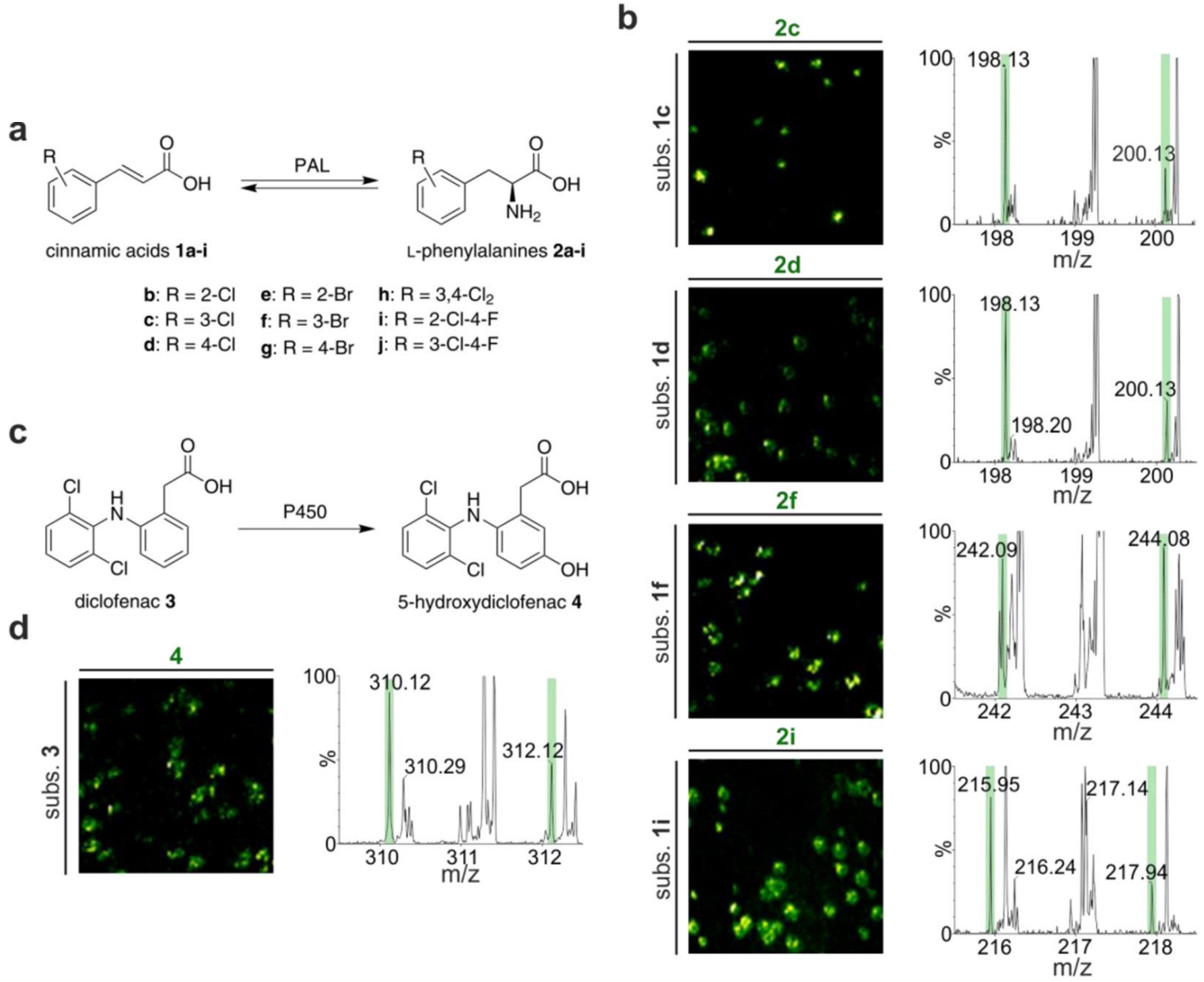


Figure 3. Application of DiBT-IMMS to screen biocatalytic reactions. (a) Expansion of the substrate scope of the PAL reaction shown in Figure 1b. (b) DiBT-IMMS imaging of the PAL reaction with different substrates, performed on colonies producing AvPAL (green: product). (c) Extension to a cofactor-dependent reaction, P450-mediated oxidation of diclofenac. (d) DiBT-IMMS imaging of the P450 reaction, performed on colonies producing P450-RhF (green: product).

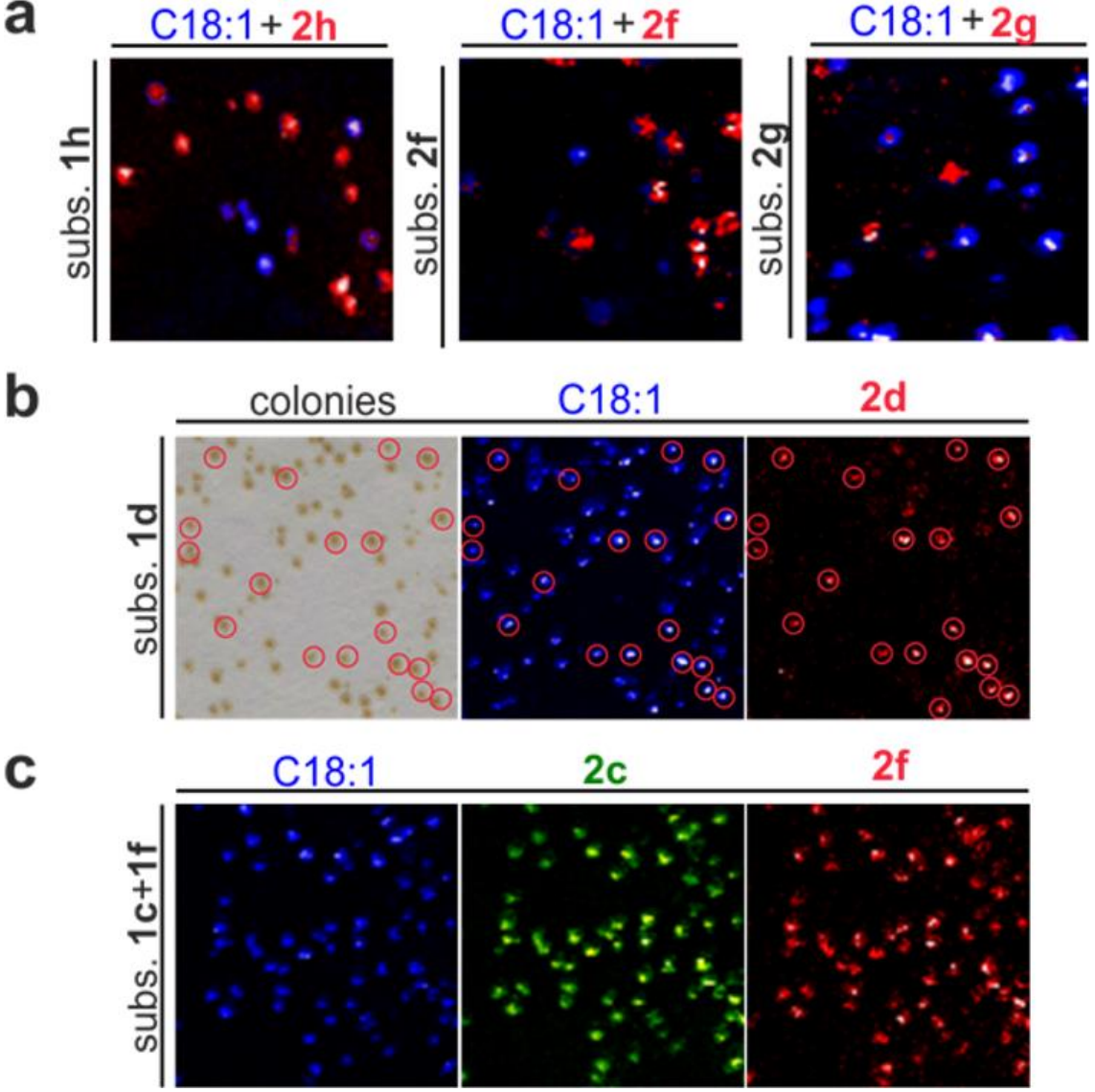


Figure 4. DiBT-IMMS imaging to screen biotransformation reactions with different colonies and/or different substrates. (a) DiBT-IMMS imaging of the PAL reaction with different substrates, performed on mixtures of colonies either producing AvPAL or harboring an empty vector, in different ratios (blue: C18:1; red: product). (b) DiBTIMMS imaging of the PAL reaction with 1d, performed on colonies either producing AvPAL or harboring an empty vector (blue: C18:1; red: product 2d), compared with the picture of the original membrane. (c) DiBT-IMMS imaging of the PAL reaction with a mixture of 1c and 1f, performed on colonies producing AvPAL (blue: C18:1; green: product 2c; red: product 2f).

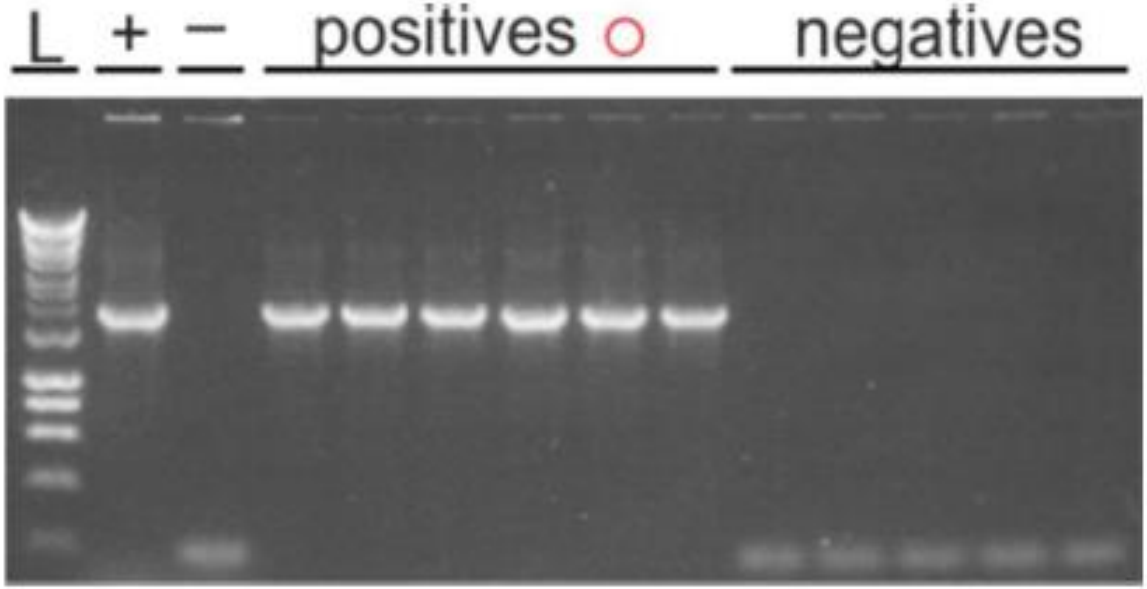
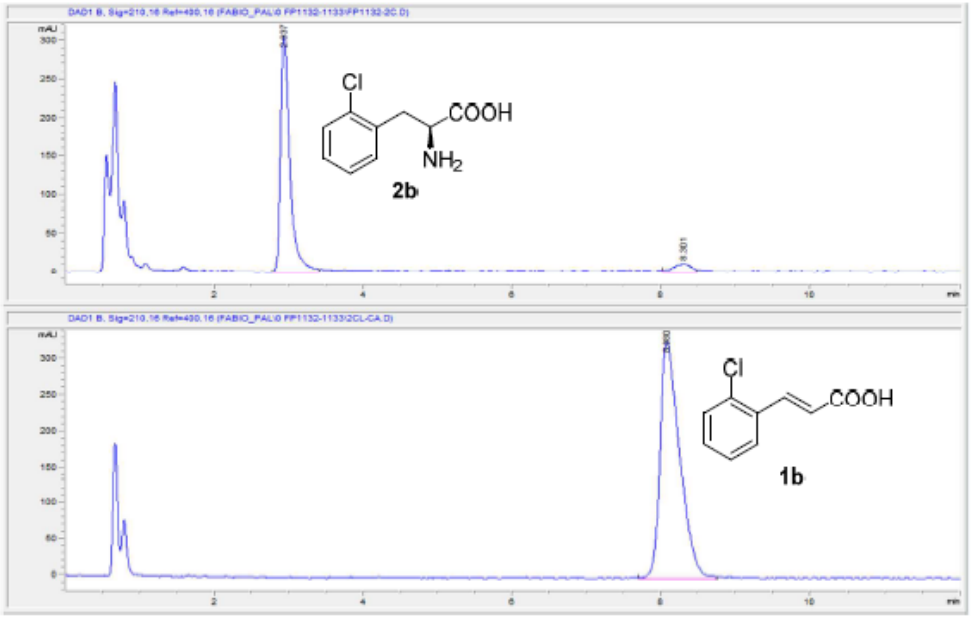
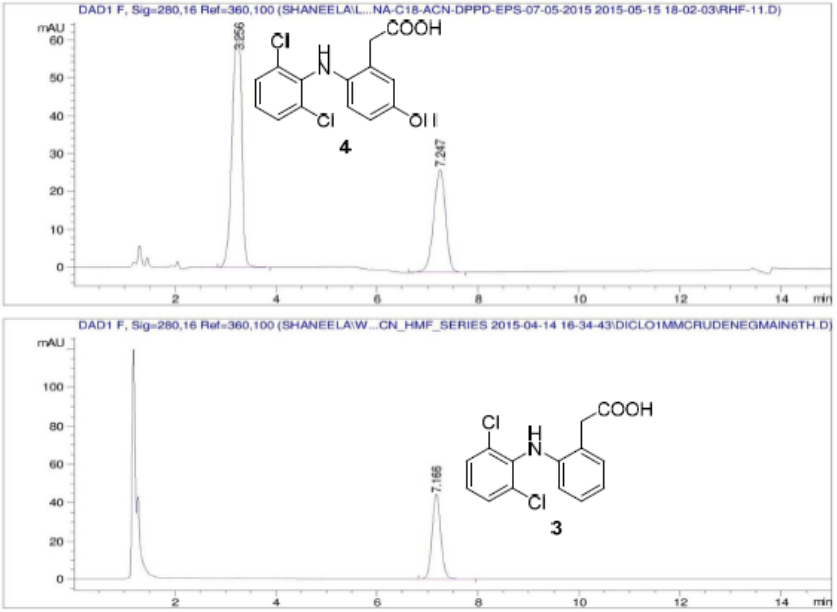


Figure S3. Colony PCR of selected positive and negative colonies after DiBT-IMMS imaging analysis using AvPAL-specific primers. Only positive colonies show the presence of the AvPAL gene. L: ladder; +: positive control (pET16b-AvPAL); –: negative control (empty pET16b); positives: colonies identified as positive from DiBT-IMMS imaging analysis; negatives: colonies identified as positive from DiBT-IMMS image analysis.





Top panels: biotransformation reaction (run in the same buffer employed for DiBT-IMMS imaging) Bottom panels: reference starting material

Conclusion

➢ They have shown for the first time that DiBT IMMS analysis of bacterial colonies from agar plates can be used for the identification of recombinant biocatalysts.

➢ This analytical method make it particularly useful for application in biocatalysis:

(i) ambient and direct sampling conditions dramatically reduce liquid handling of samples and allow for in situ extraction of DNA;
(ii) activity measurements are label-free, thus allowing for universal workflows matching the need to screen many different biocatalytic reactions and libraries of potential substrates;
(iii) high resolution of activity measurements by mass allows for multiplexing biocatalytic reactions to match the need for combinatorial sampling (substrate vs variant libraries);
(iv) ambient sampling means that biotransformations can be monitored in real time in live colonies.

➢The potential capabilities of this semi in vivo analytical method enable the directed evolution library screening by integrating the DNA information with analysis results.

Thank you