Paper Presentation

- **Pallab Basuri**

10/03/2018

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

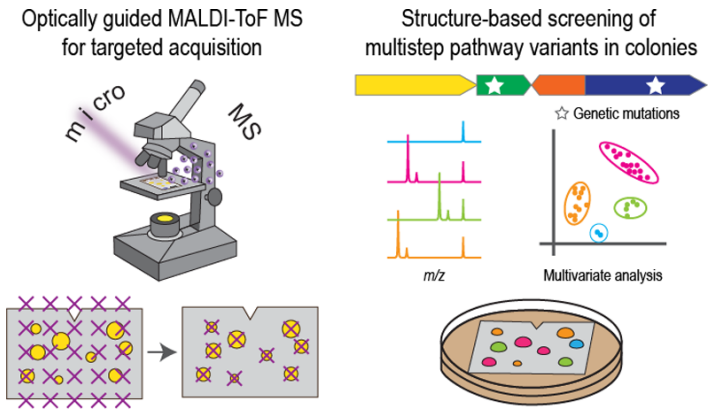
pubs.acs.org/JACS

Profiling of Microbial Colonies for High-Throughput Engineering of Multistep Enzymatic Reactions via Optically Guided Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

Tong Si,[†,¶] Bin Li,[‡,§,∥,¶] Troy J. Comi,[‡,§,¶] Yuwei Wu,[‡] Pingfan Hu,[⊥] Yuying Wu,[⊥] Yuhao Min,[‡] Douglas A. Mitchell,[†,‡,#] Huimin Zhao,[*,†,‡,⊥,∇,⊗] and Jonathan V. Sweedler[*,†,‡,§]

[†]Carl R. Woese Institute for Genomic Biology, [‡]Department of Chemistry, [§]Beckman Institute for Advanced Science and Technology, [⊥]Department of Chemical and Biomolecular Engineering, [#]Department of Microbiology, [∇]Department of Biochemistry, [⊗]Department of Bioengineering, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States

[∥]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China



Published: August 9, 2017
DOI: 10.1021/jacs.7b04641
J. Am. Chem. Soc. 2017, 139, 12466−12473

Background of the work-

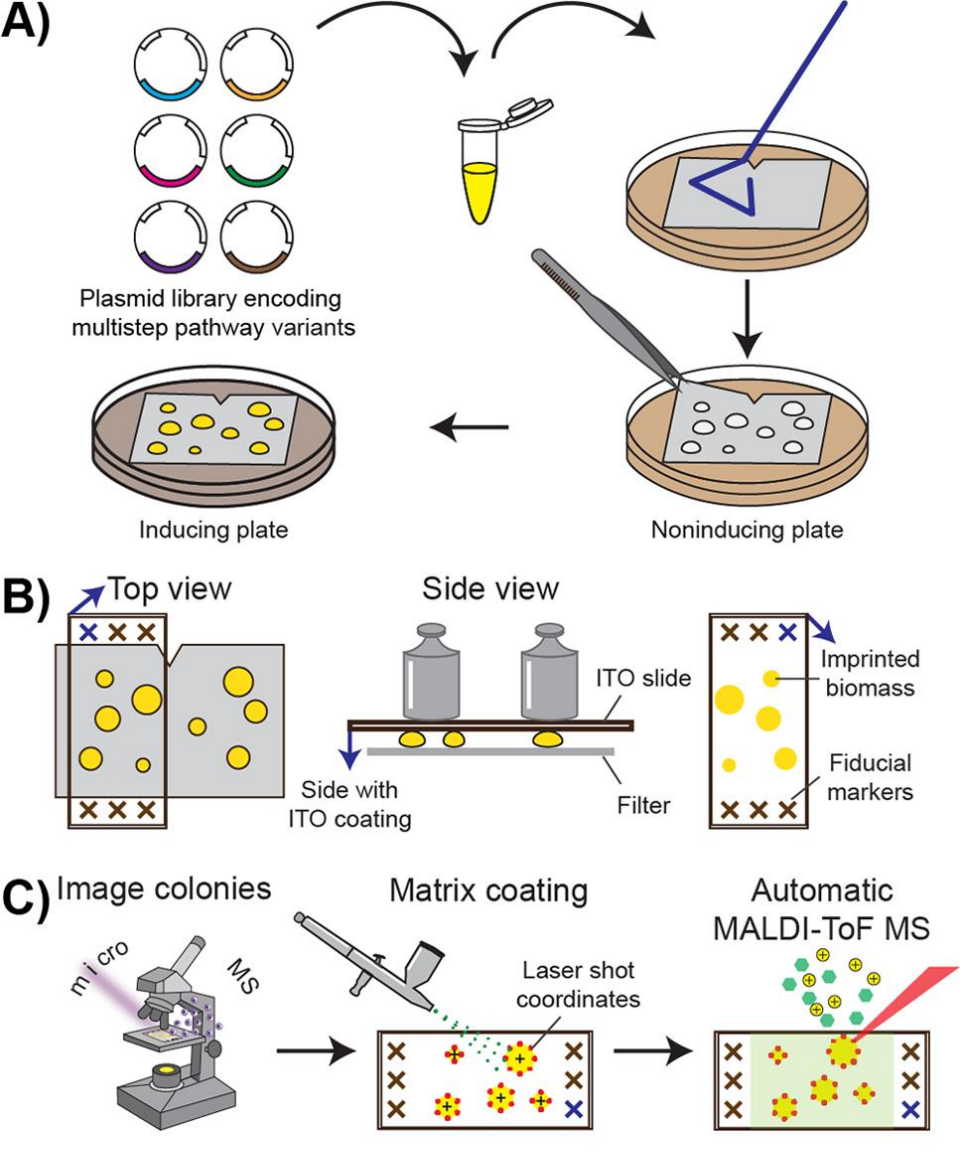
1) Mass spec imaging of bacterial colony for biotransformation of molecules is a subject area which has been studying for last couple of years. And some number of work based on DESI imaging has already been done.

Why this paper was chosen-

1) This particular paper was written in the form where MALDI was used as a eye of the observer for rapid screening of the single or double step biotransformation in a branch of genetically modified species.
2) Correlating the cluster analysis of huge number of spectrum with the optical image to see the relative abundance of a molecule in a particular genetically modified bacterial colony is something industrially important.

Group relevancy-

1) As a mass spec prospective our HR MS can be utilized to analyze this kind of work in addition to desi or nanodesi imaging.
2) Rapid screening of material and cells interaction e.g MoS_2 or Arsenic .
3) Genetically modified cells which will produce excess food supplementary can be used a printing material to produce printed food, e.g printed protein-bar.



In this paper ....

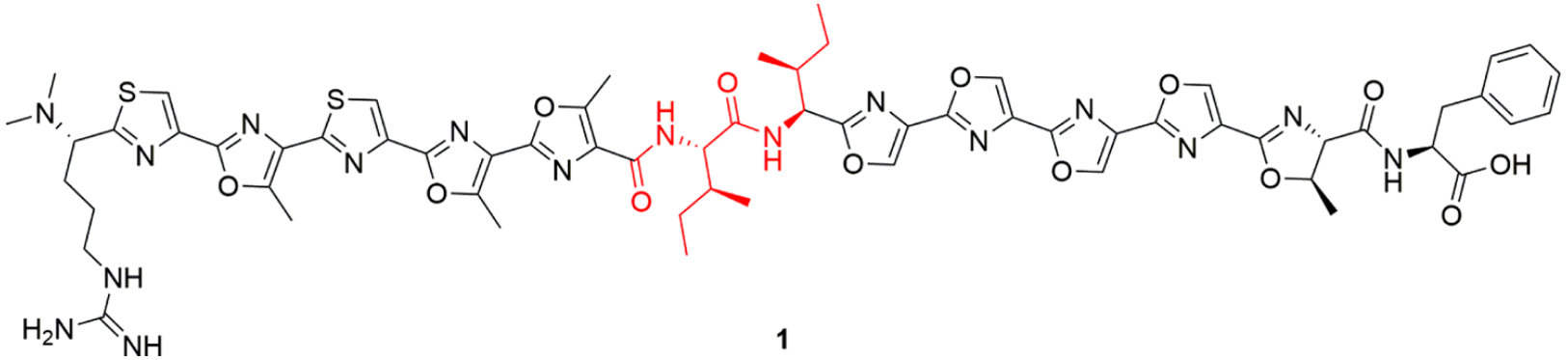
Figure 1. Optically guided MALDI-ToF MS screening.

(A) Strain library preparation.

(B) Imprinting of a colony biomass onto indium tin oxide (ITO)-coated glass slides (blue arrows indicate the coating side).

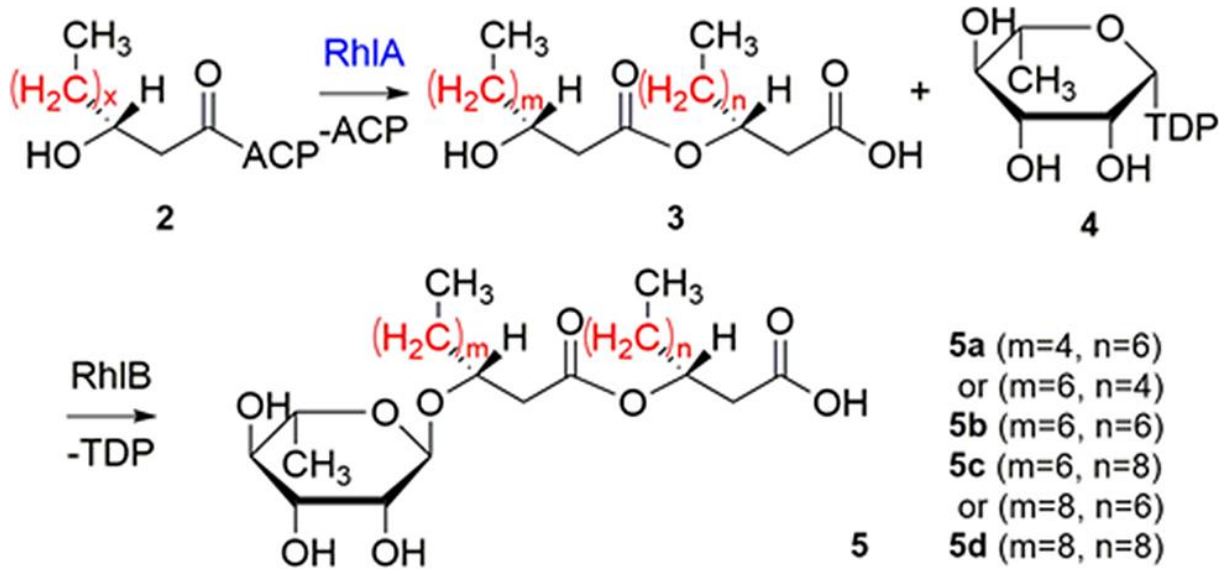
(C) Generation of laser coordinates for automated MALDI-ToF MS profiling using machine vision on microscopic images.

(D) Multivariate analysis and visualization of resulting mass spectra data sets.



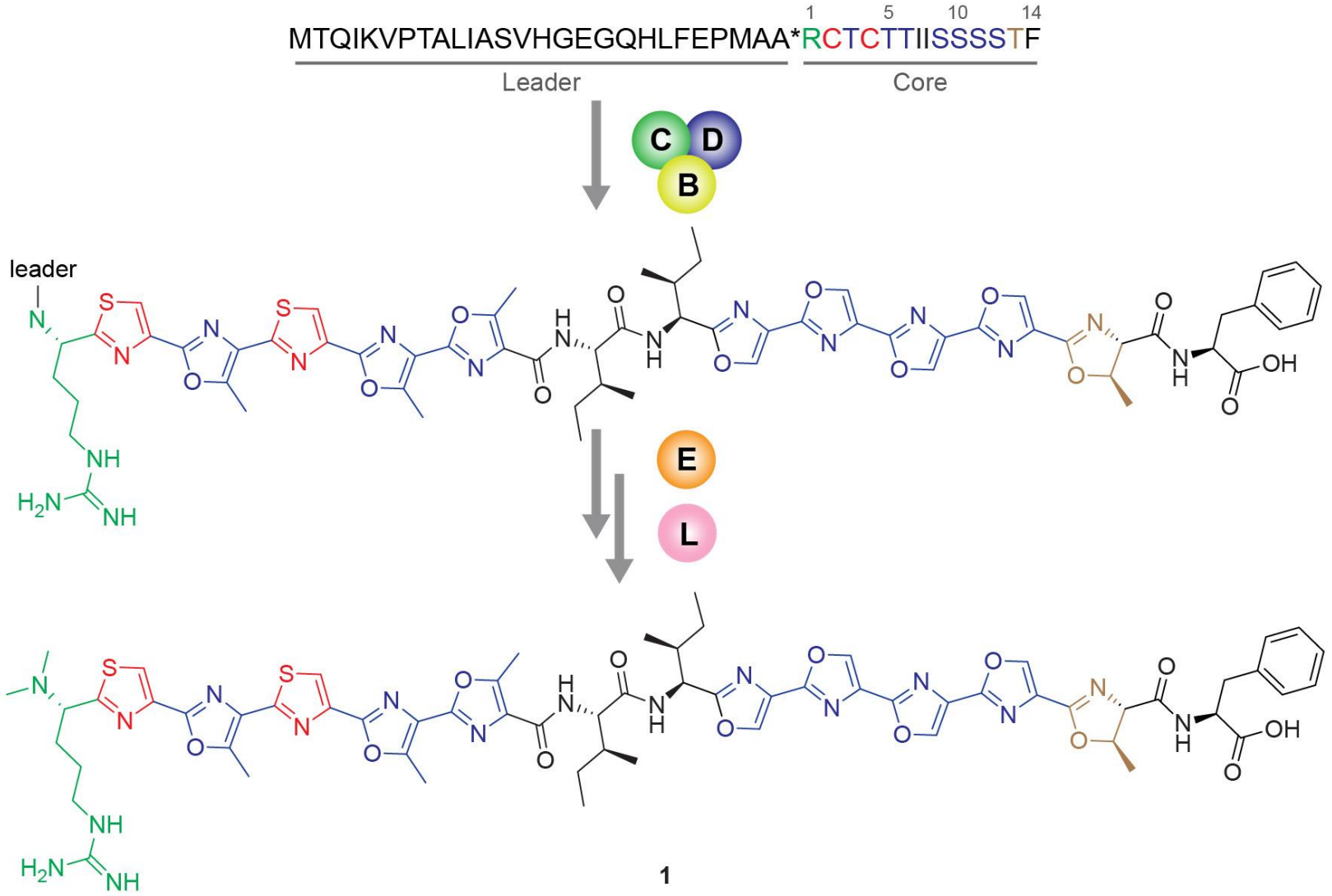
1

Scheme 1. Structure of PZN 1

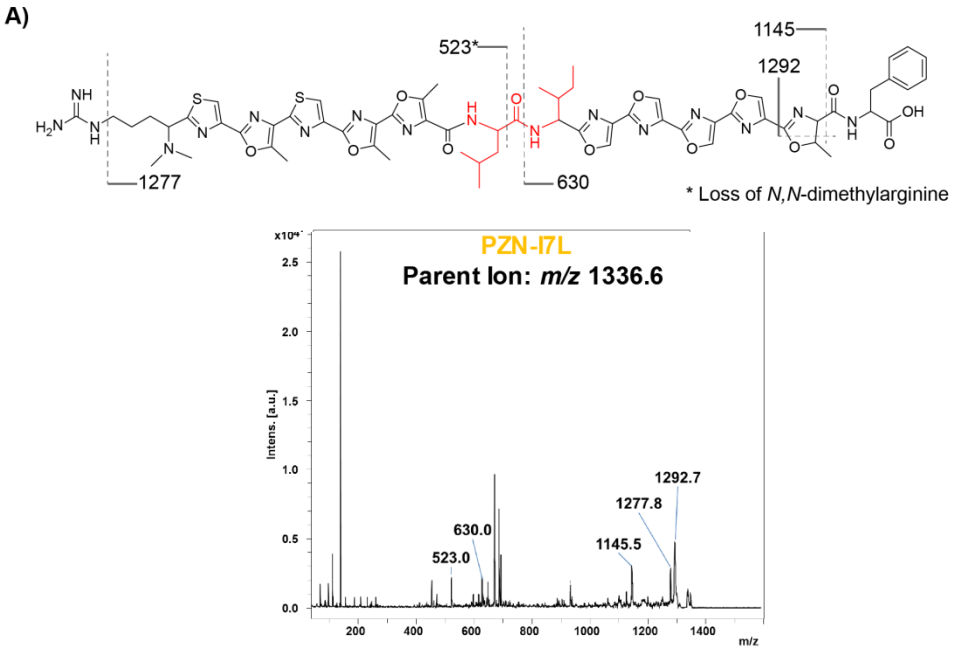


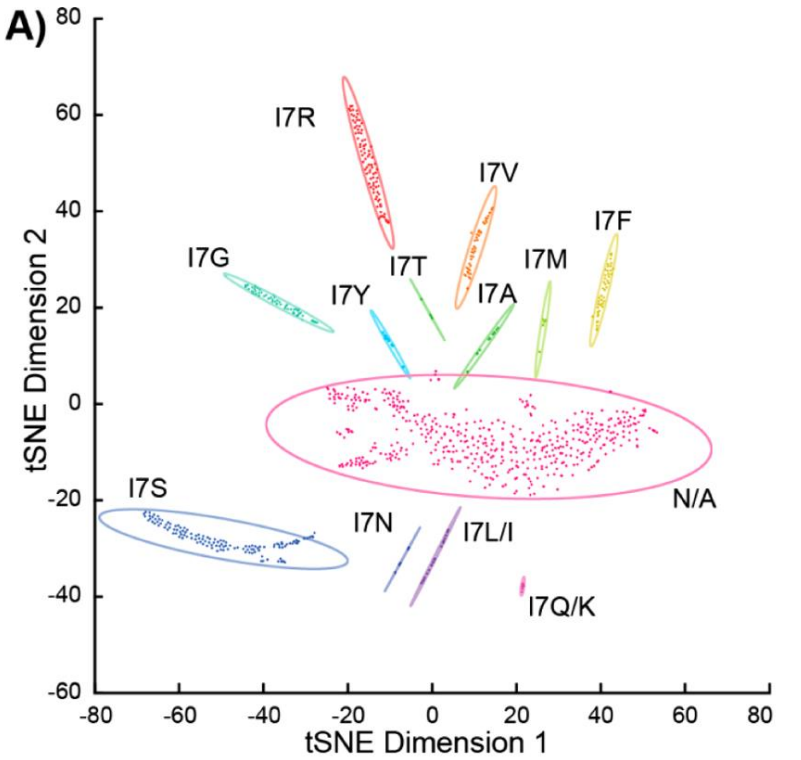
2 3 4 5

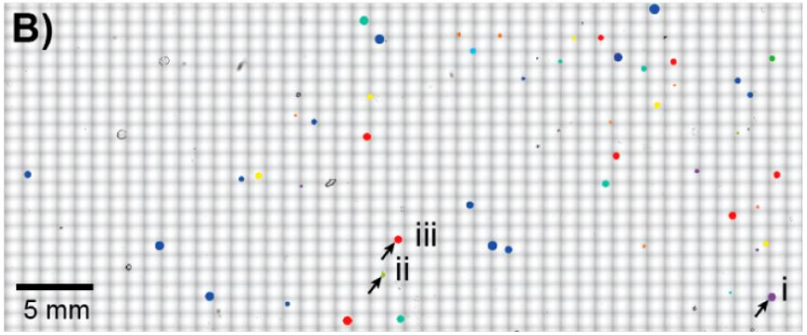
Scheme 2. Biosynthesis of RL 5



Scheme S1. Main biosynthetic steps of PZN 1. The leader cleavage site in the precursor peptide is denoted by the asterisk. The main biosynthetic enzymes include a trimeric heterocycle synthetase consisting of a dehydrogenase (B) and two cyclodehydratase (C/D), a putative leader peptidase (E), and a methyltransferase (L). (Adapted from ref.8 with permission. Copyright 2017 American Chemical Society).







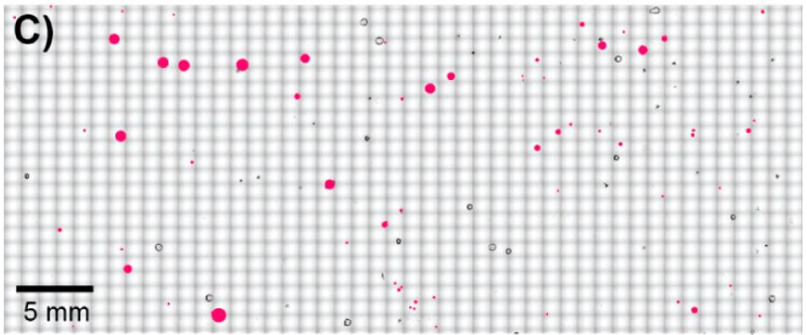
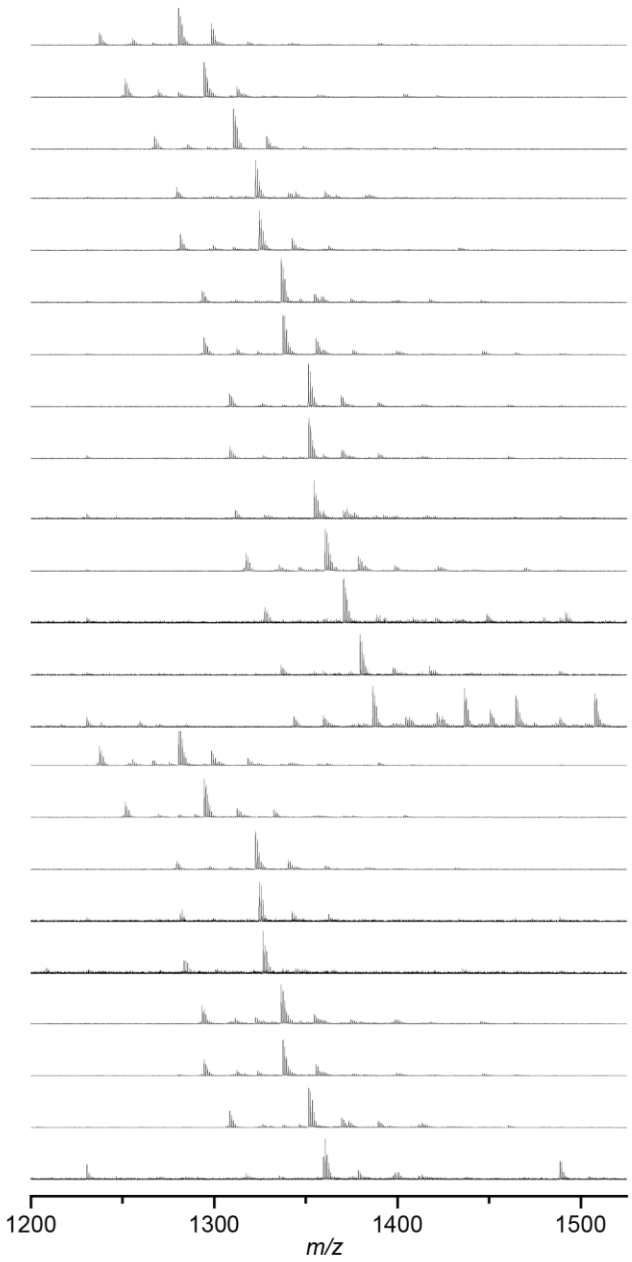
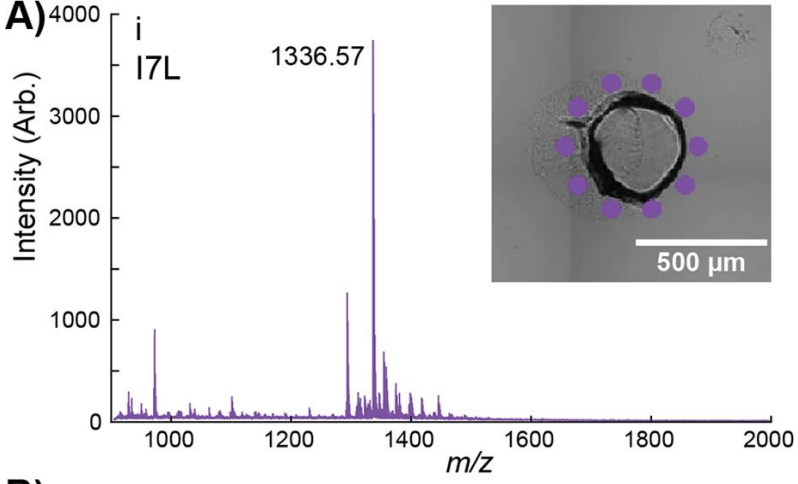


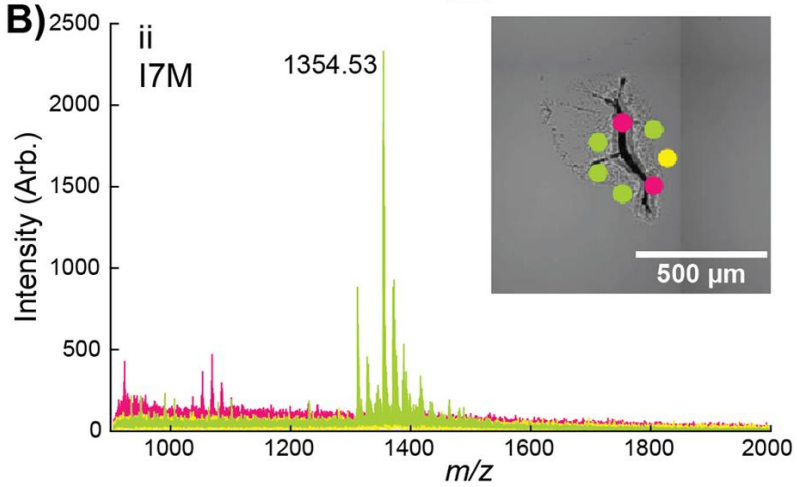
Figure 2. Multivariate analysis of PZN analogues. (A) Visualization with targeted t-SNE clustering of the I7 library from a single experiment. Each point corresponds to a single mass spectrum, with each cluster surrounded by a 95% confidence ellipsoid. The N/A cluster contains spectra without observable peptide signals. The position of (B) each mutant or (C) N/A colony is mapped onto the optical image to aid mutant recovery. The three colonies highlighted in panel B are displayed in more detail in Figure 3.



AA mutation by MS	Theoretical monoisotopic *m/z* $[M+H]^+$	TOF *m/z* $[M+H]^+$	FT-ICR *m/z* $[M+H]^+$	FT-ICR mass error (ppm)	DNA sequencing	
					Codon	AA
I7G	1280.41490	1280.452			GGT	I7G
I7A	1294.43055	1294.469			GCT	I7A
I7S	1310.42546	1310.541	1310.42424	-0.93	AGT	I7S
I7V	1322.46185	1322.528	1322.46206	0.16	GTT	I7V
I7T	1324.44111	1324.544	1324.44063	-0.37	ACG	I7T
I7L	1336.47750	1336.590	1336.47823	0.55	CTG	I7L
I7N	1337.43636	1337.512			AAT	I7N
I7Q	1351.45201	1351.389	1351.45219	0.13	CAG	I7Q
I7K	1351.48840	1351.571	1351.48848	0.06	AAG	I7K
I7M	1354.43392	1354.522	1354.43408	0.12	ATG	I7M
I7H	1360.45235	1360.441			CAT	I7H
I7F	1370.46185	1370.587			TTT	I7F
I7R	1379.49455	1379.576	1379.49482	0.20	AGG	I7R
I7Y	1386.45676	1386.520	1386.45743	0.48	TAT	I7Y
I8G	1280.41490	1280.521	1280.41506	0.13	GGG	I8G
I8A	1294.43055	1294.533			GCT	I8A
I8V	1322.46185	1322.497			GTT	I8V
I8T	1324.44111	1324.544	1324.44065	-0.35	ACT	I8T
I8C	1326.40262	1326.520			TGT	I8C
I8I	1336.47750	1336.536			CTT	I8L
I8N	1337.43636	1337.538			AAT	I8N
I8Q	1351.45201	1351.510	1351.45201	0.81	CAG	I8Q
I8H	1360.45235	1360.596			CAT	I8H

Figure S1. Summary of PZN 1 analogues observed in this study. For each PZN 1 analogue, a typical MALDI-ToF mass spectrum of the *E. coli colony is included on the left. The table on the right* summarizes the information of PZN 1 analogues including the amino acid (AA) mutation assignment based on mass spectra, theoretical and measured monoisotopic *m/z value of the [M+H]+ ion (by MALDIToF* or MALDI-FT-ICR), and DNA sequencing results. High-resolution FT-ICR was performed on select colonies from the same sample target subsequent to MALDI MS screening, focusing on tentative Q and K mutants, as well as select 1 analogues (shown in red) not reported in a previous study.





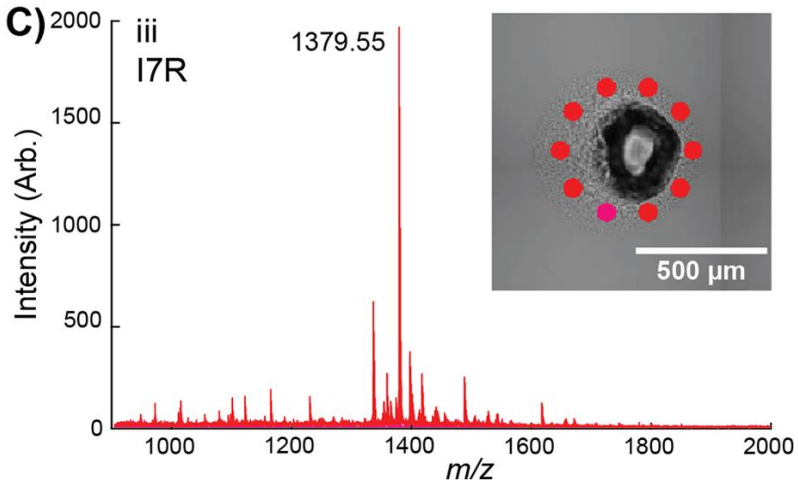


Figure 3. Detailed view of annotated colonies (i, ii and iii) from Figure 2 shown in panels (A−C) respectively. The color of each cluster corresponds to the t-SNE plot in Figure 2. Average spectra of each cluster for the colony are displayed with the base peak labeled. Mutations were confirmed by DNA sequencing (Figure S1).

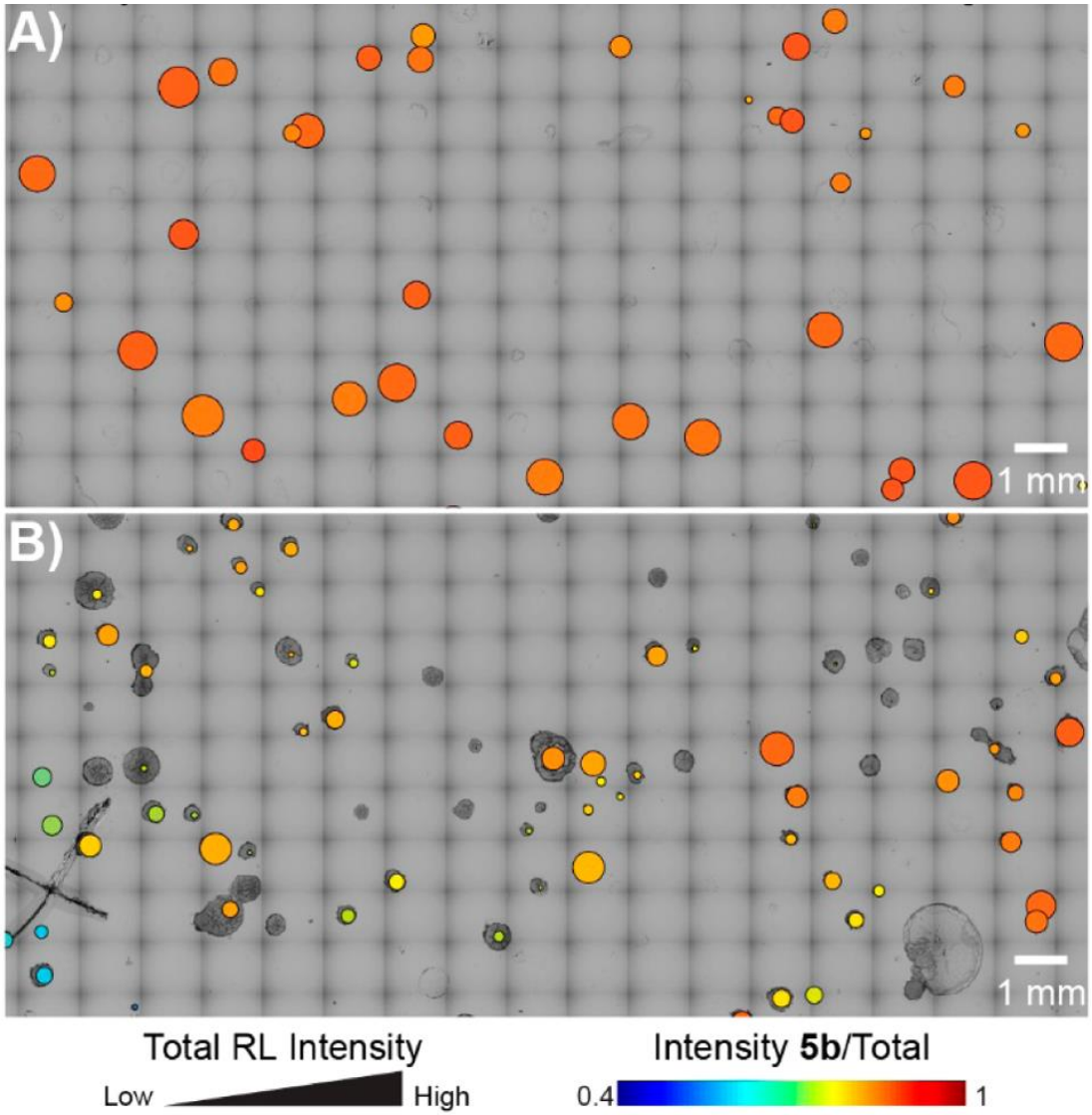


Figure 4. Visualization of MALDI-ToF MS screening results of mono- RL-producing E. coli colonies. (A) WT. (B) Strain library in the first round of mutagenesis (R1). For each circle overlaying the corresponding colony, the radius scales with the log-base 10 intensity of the sum of all RL peaks, and the false color scales with the relative abundance of RL 5b. Only a small, representative region is shown.

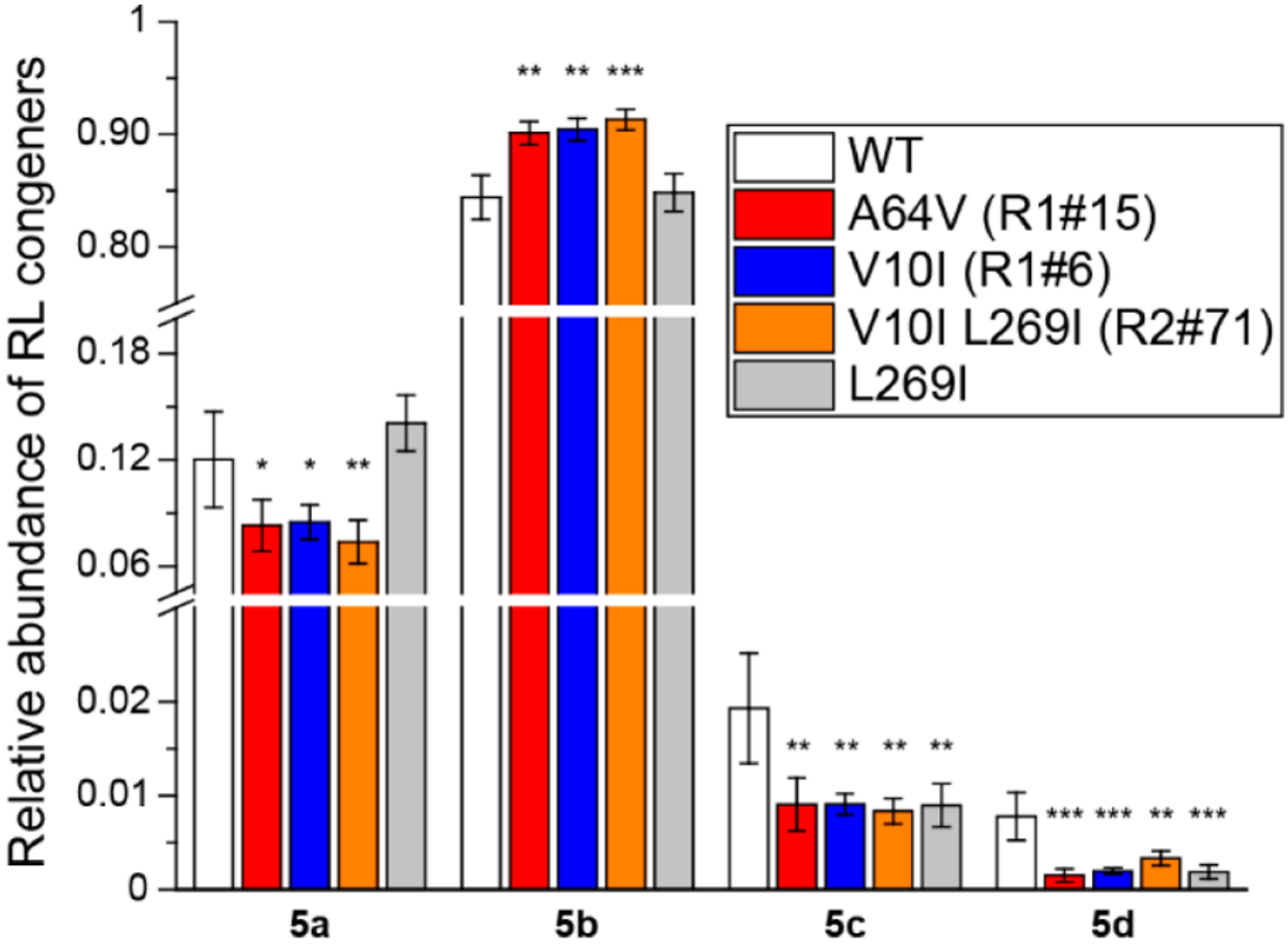


Figure 5. Comparison of RL production in liquid cultures between WT and isolated mutant strains quantified using LC−MS/MS in multiple reaction monitoring mode. Error bars indicate the standard deviations of biological triplicates. Significant differences were determined between WT and mutants using an independent two tailed, two-sample t-test for equal sample sizes and equal variance. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Conclusion-

1) Successfully demonstrated optically guided MALDI MS screening of large number of bacterial colonies.

2) Incorporating machine vision and automatic target patterning greatly improves MS acquisition efficiency over traditional MSI assays, especially for randomly distributed colonies.

3) Correlating genotype and the phenotype using MSI is an interesting approach.

Thank you