

Development of Substrates That Do Not Leak from Droplets

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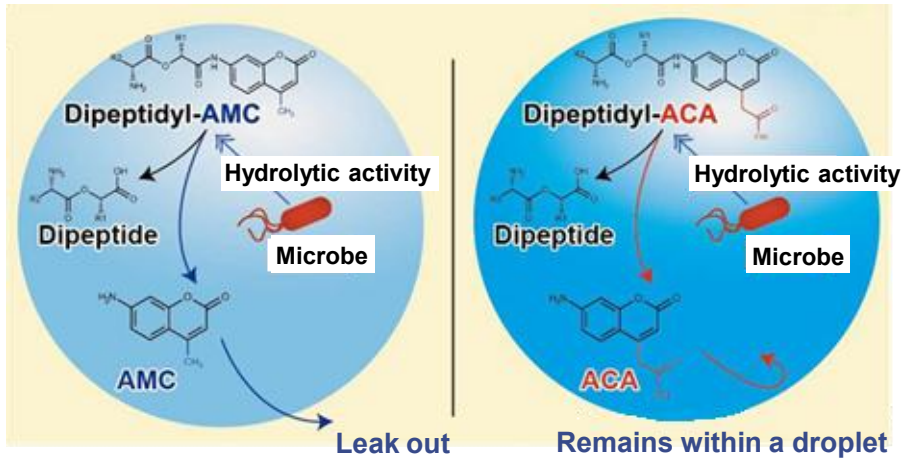
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- Overview -

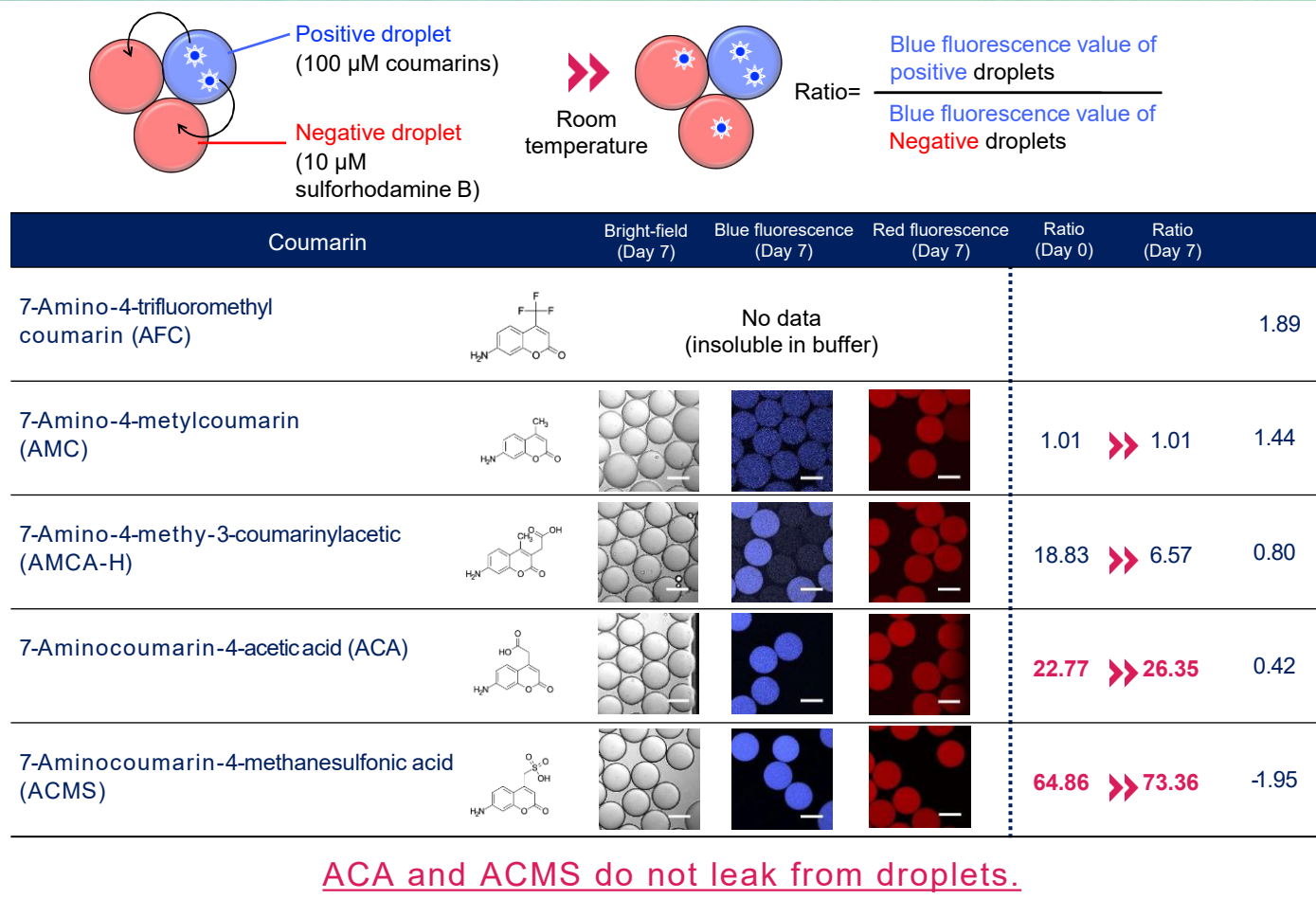
Screening techniques combining droplets and microfluidic devices have attracted attention as tools for ultra-efficient biochemical assays and microbial exploration. In particular, water-in-oil (w/o) droplets with volumes of nL to pL using fluorinated oils as the oil phase have been used for microbial culture and as a site for enzymatic reactions in liquid. Since w/o droplets are compartmentalized by surfactants, substances contained within the droplets may migrate between droplets depending on the substances' properties. This phenomenon can be a barrier to the detection of enzymatic reactions inside the droplets. For example, 7-amino-4-methylcoumarin (AMC), a derivative of coumarin used as a fluorescent substrate for various compounds such as peptidases/proteases, leaks out from w/o droplets due to its high hydrophobicity. Therefore, AMC has not been used in screening technique involving w/o droplets. In this study, we searched for coumarin compounds that do not leak from w/o droplets and subsequently developed new substrates based on these compounds. In addition, we screened 160,000 droplets at a rate exceeding 100 droplets per second using the developed substrates and detected environmental microbes with peptidase activity, thereby confirming the utility of the droplets. The present study has succeeded in greatly expanding the number of detectable enzyme species in microfluidic systems using w/o droplets. (Reference: A. Nakamura et al., *Anal. Chem.*, 94 (5), 2416–2424, 2022)

Conventional Study

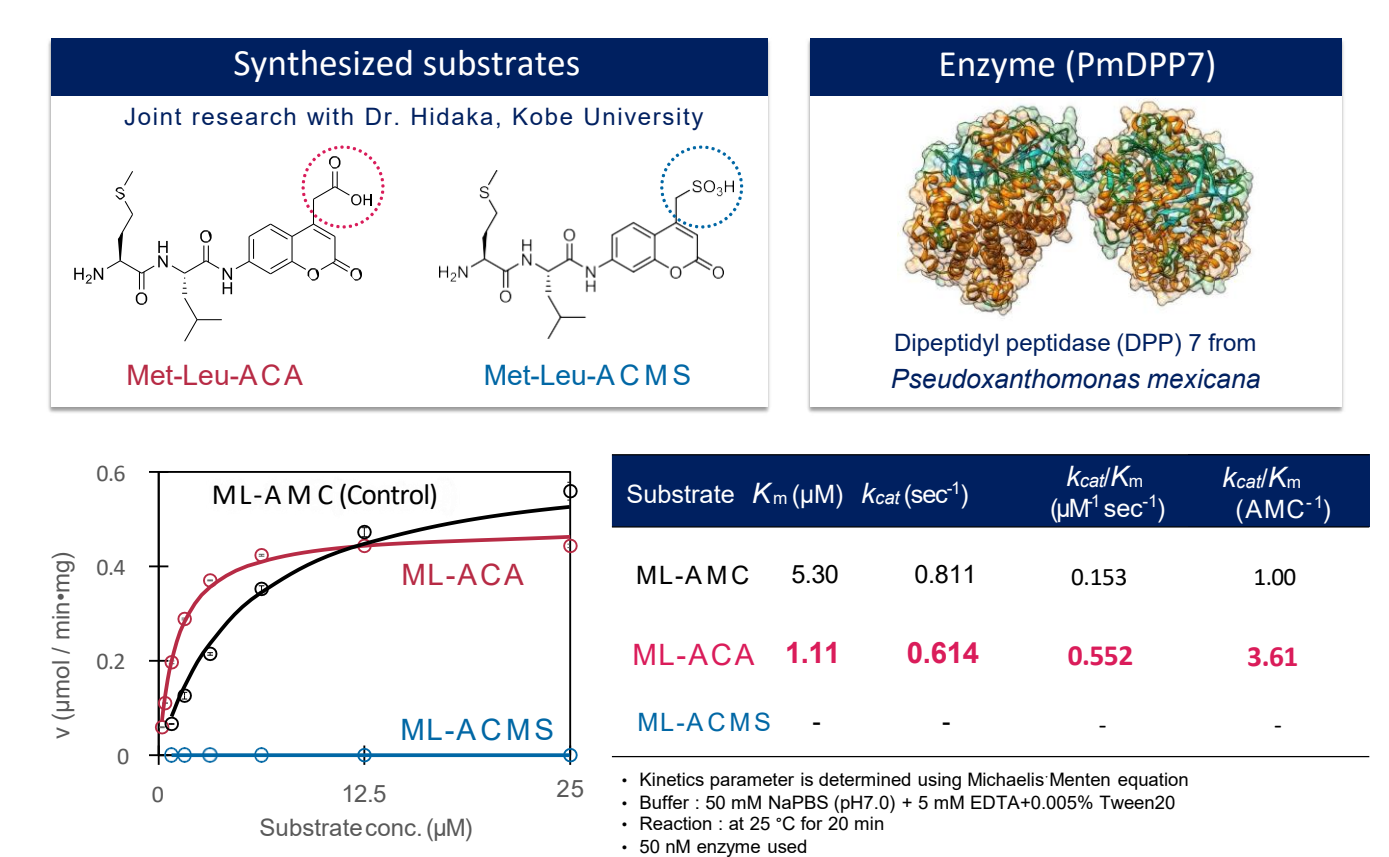


- Results and Discussion -

1. Search for coumarin compounds that do not leak



2. Development of ACA- and ACMS-based substrates



Activity was detected with the ACA substrate.

■ Verification of activity of four enzymes using two substrates (Met-Leu-ACA and Leu-Asp-ACA)

Enzymes	Substrates	K_m (μM)	k_{cat} (sec ⁻¹)	k_{cat}/K_m (μM ⁻¹ sec ⁻¹)	k_{cat}/K_m (AMC ⁻¹)
PmDPP7	Met-Leu-AMC	5.30	0.811	0.153	1.00
	Met-Leu-ACA	1.11	0.614	0.552	3.61
SmDPP7	Met-Leu-AMC	21.1	0.849	0.0402	1.00
	Met-Leu-ACA	4.09	0.647	0.158	3.94
PgDPP11	Leu-Asp-AMC	10.6	5.22	0.492	1.00
	Leu-Asp-ACA	10.1	3.90	0.386	0.785
SmDPP11	Leu-Asp-AMC	45.0	18.0	0.401	1.00
	Leu-Asp-ACA	12.2	22.8	1.88	4.68

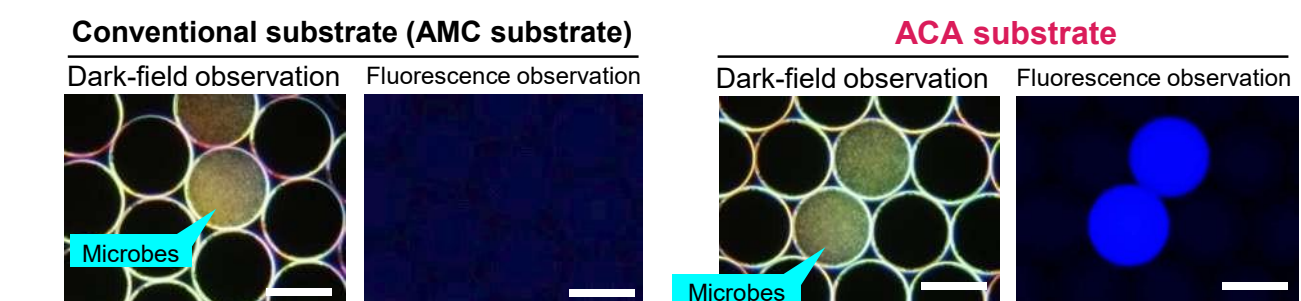
• Synthesized by Dr. Hidaka, Kobe University
• Kinetics parameter is determined using Michaelis-Menten equation
• Reaction : at 25 °C for 20 min
• Buffer : 50 mM NaPBS (pH7.0) + 5 mM EDTA+0.005% Tween20

• PmDAPBII: DPP7 from *Pseudoxanthomonas mexicana* WO24
• SmDPP7: DPP7 from *Stenotrophomonas maltophilia* K279a
• PgDPP11: DPP11 from *Porphyromonas gingivalis* W83
• SmDPP11: DPP11 from *S. maltophilia* K279a

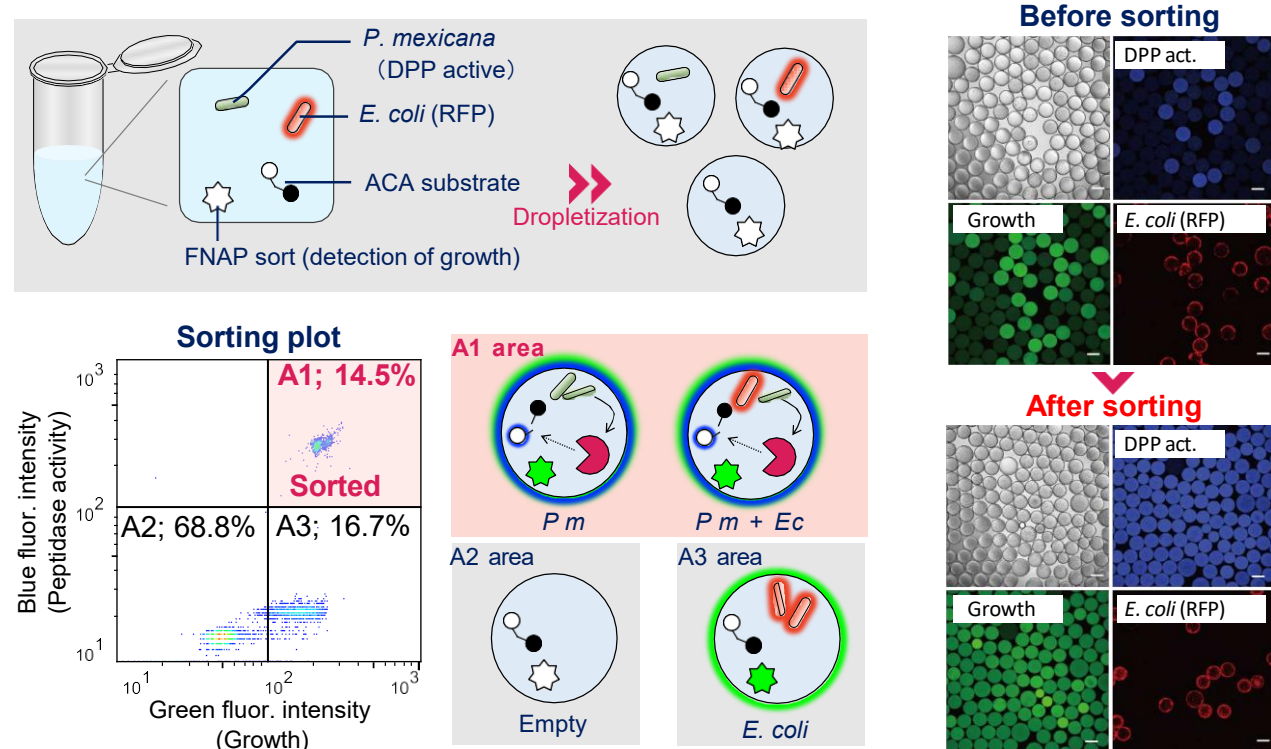
The activity of all four enzymes tested in this study was successfully detected using the ACA substrates.

3. Isolation of a model microbe using the ACA substrate

■ Detection of microbial (*P. mexicana*) cell activity inside droplets

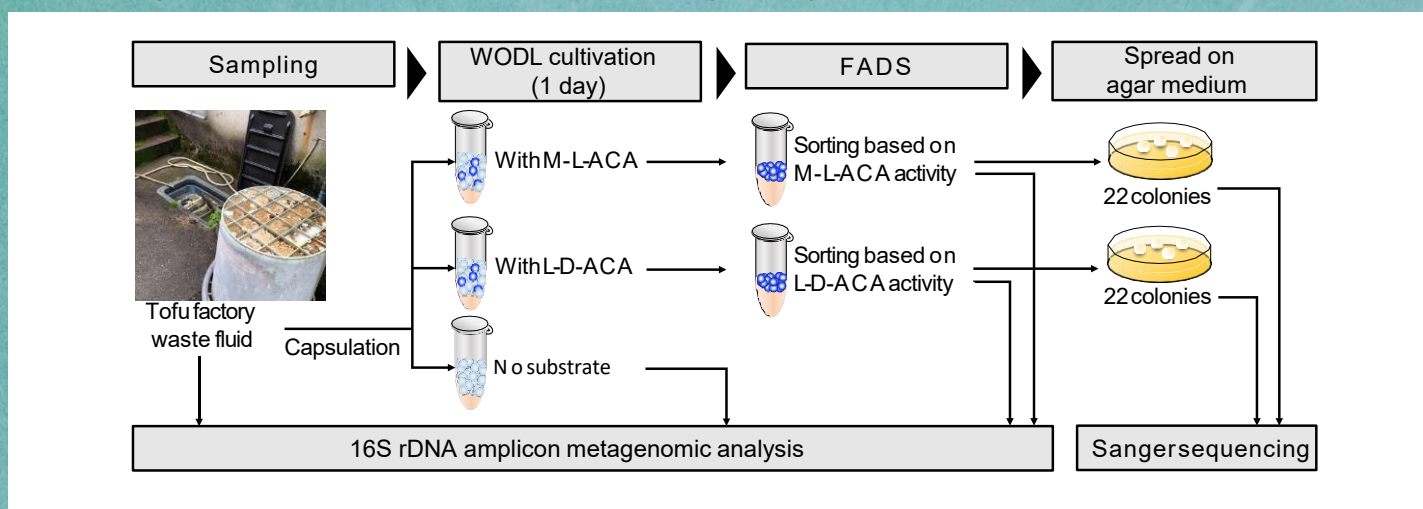


■ Isolation of microbial cells from a mixed solution of two microbial species

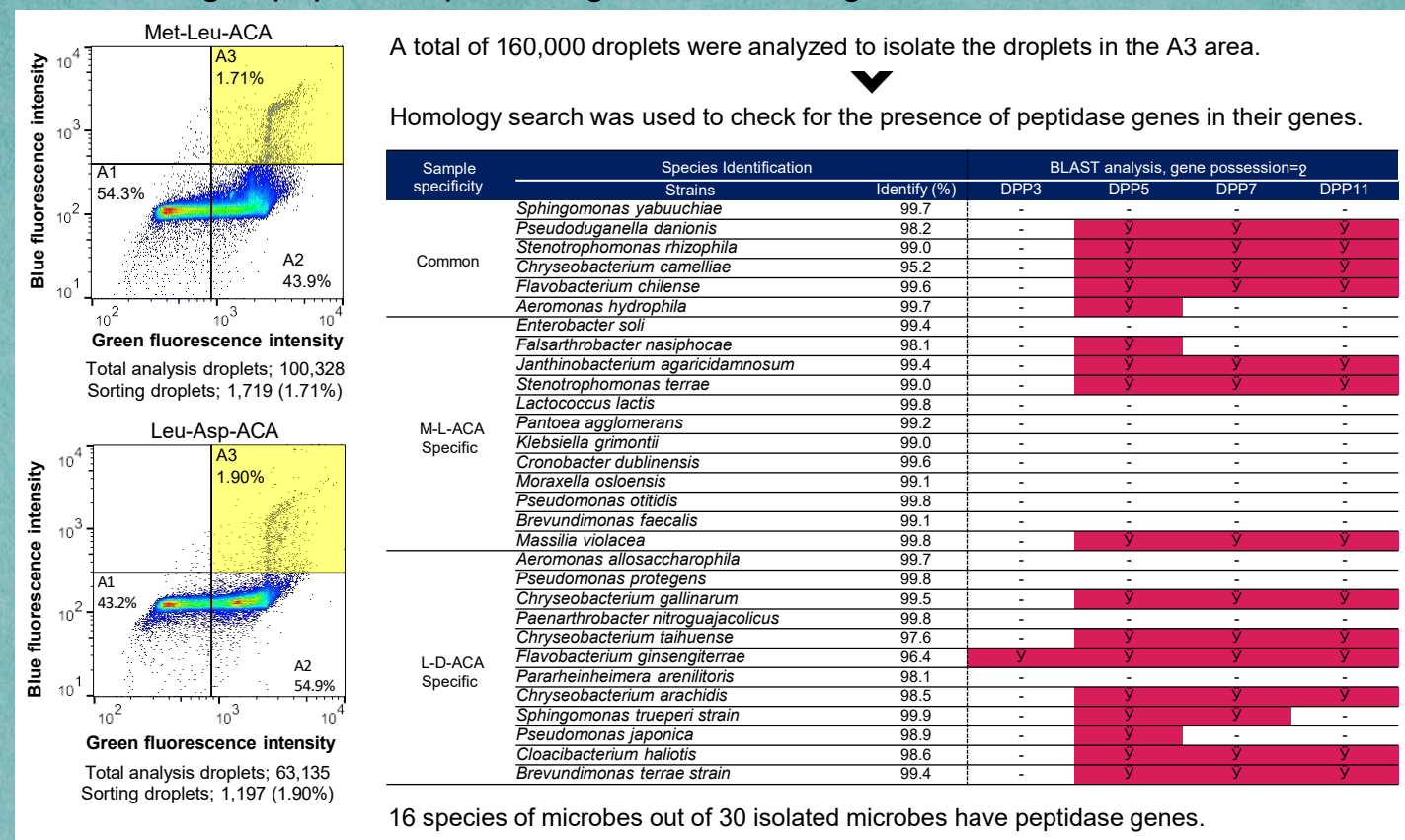


Isolated the model microbe based on peptidase activity.

4. Strategic scheme for microbial screening using ACA substrates



5. Screening of peptidase-producing microbes using ACA substrates



Conclusion

- A coumarin-derived 7-amino-4-acetic acid (ACA) substrate, which can be used in droplets, was developed, and its applicability to high-throughput screening of environmental microbes was successfully verified.
- The results of this study expand the number of detectable enzyme species in microfluidic systems using w/o droplets.