

Single-cell rapid identification, in situ viability and vitality profiling, and genome-based source-tracking for probiotics products

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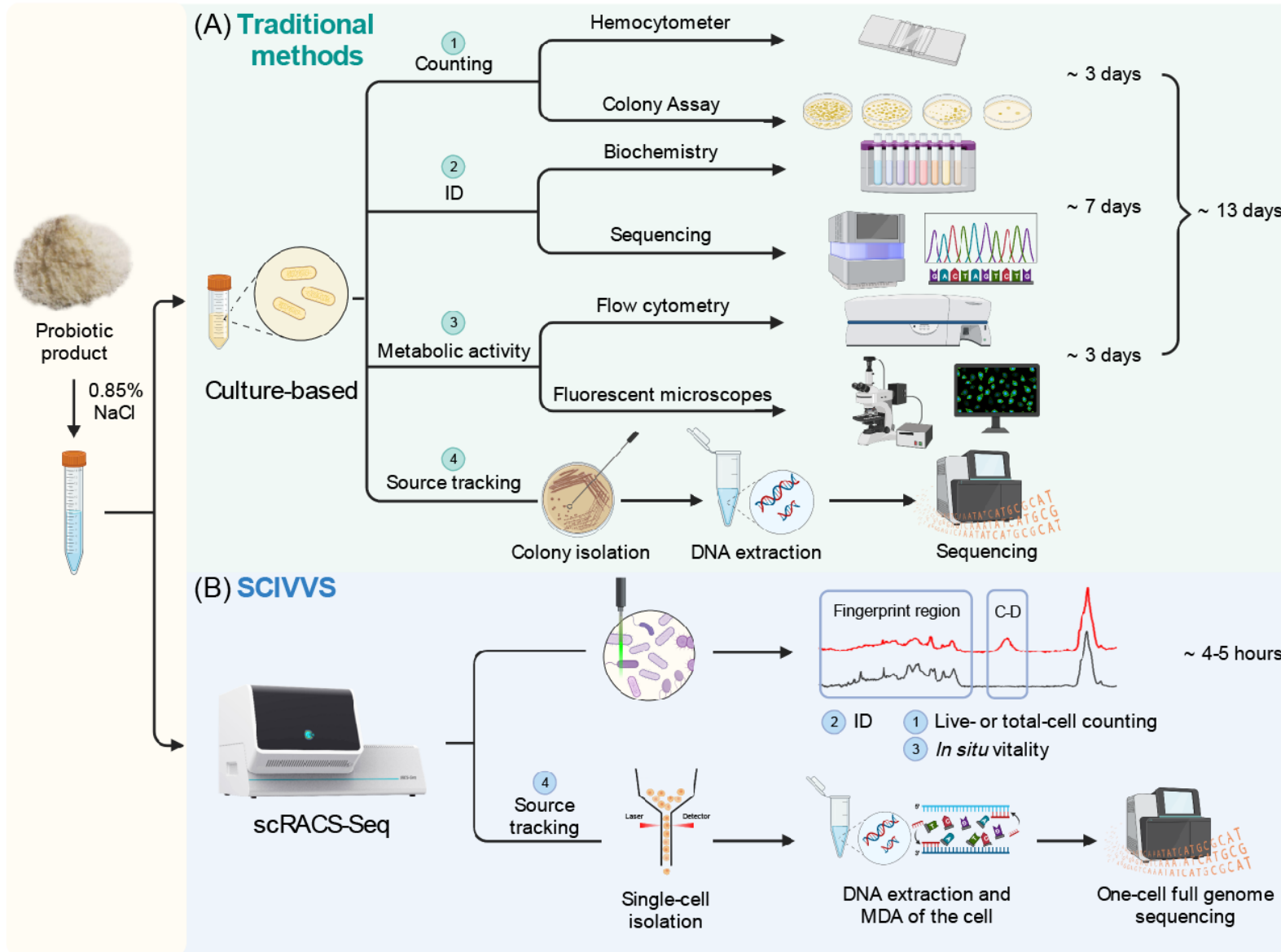
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The SCIVVS workflow for total live-bacteria count, rapid identification, species-resolved in situ vitality test, and source-tracking at precisely one-cell resolution directly from commercial probiotic products.



Traditional method
Single "colony" accuracy

Limitations: relies on pure culture, time-consuming, unable to measure "in situ viability", difficult to achieve quality control of complex probiotic products, not easy to integrate.

SCIVVS

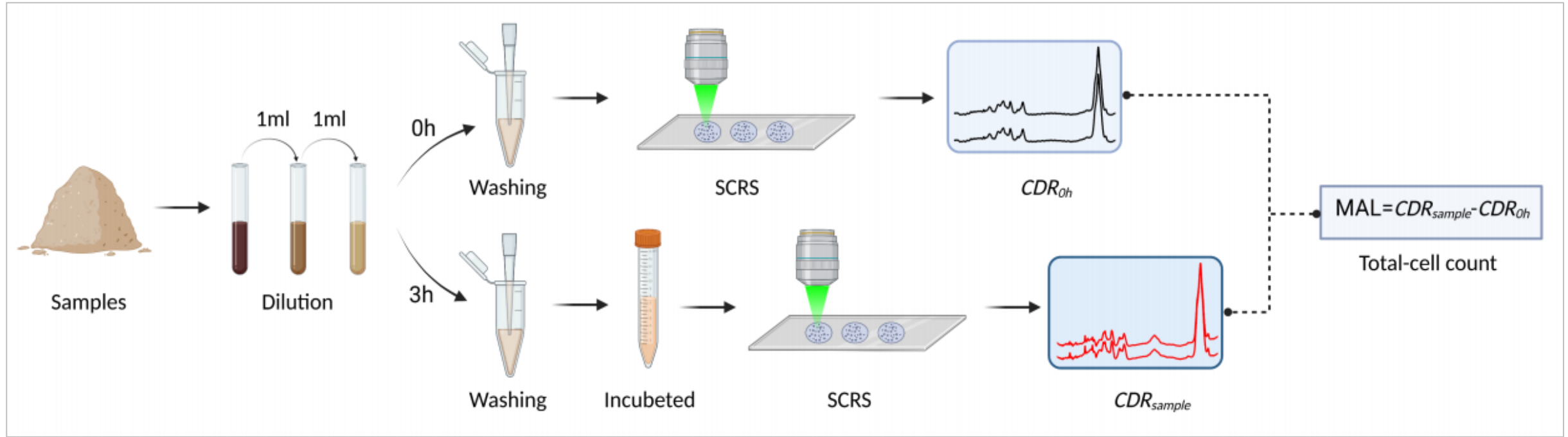
(Single-Cell Identification, Viability & Vitality tests and Source-tracking)

Single "cell" accuracy

Advantages: fast, sensitive, comprehensive, and low-cost strategies, and an integrated single-cell SCIVVS workflow for compound probiotic product quality assessment .



The workflow for determining “Metabolic Activity Level” (MAL) as the basis for the vitality test



MAL: Metabolic Activity Level

MAL ≤ 0, dead cells

MAL > 0, live cells

rMAL: relative Metabolic Activity Level

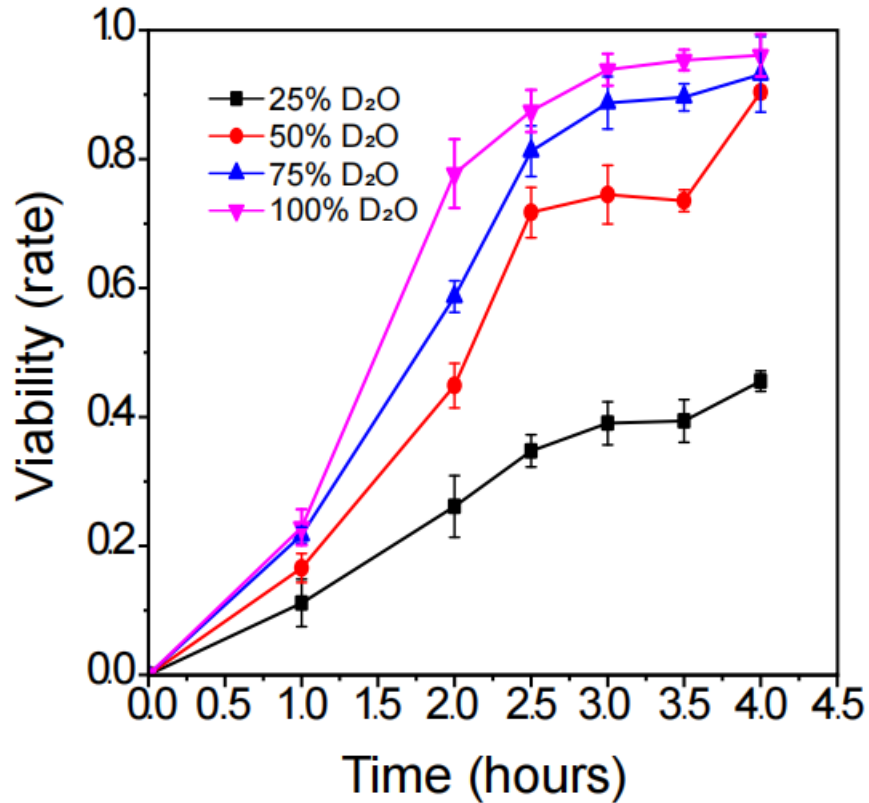
Tao, et al, Metabolic-activity based assessment of antimicrobial effects by D2O-labeled Single-Cell Raman Microspectroscopy, *Anal Chem*, 2016

$$rMAL = \frac{CDR_{sample} - CDR_{0h}}{CDR_{control} - CDR_{0h}}$$



Determination of experimental conditions based on SCRS live-bacteria counting

Different concentrations of D₂O at different times



75% or 100% D₂O: 3 h

Plate count results under 75% and 100% D₂O

	Plate count (CFU/g)		P value
	0 hours	3 hours	
75% D ₂ O	$1.27 \pm 0.10 \times 10^{10}$	$2.81 \pm 0.09 \times 10^{10}$	H ₁ : $p < 0.05$
100% D ₂ O	$1.26 \pm 0.03 \times 10^{10}$	$1.31 \pm 0.05 \times 10^{10}$	H ₀ : $p > 0.05$

Compared to the culture method:

- 75% D₂O, skew the total-cell and live-cell counting results
- 100% D₂O, no significant difference

Incubation in 100% D₂O for 3 hours was determined as the conditions for sample preparation



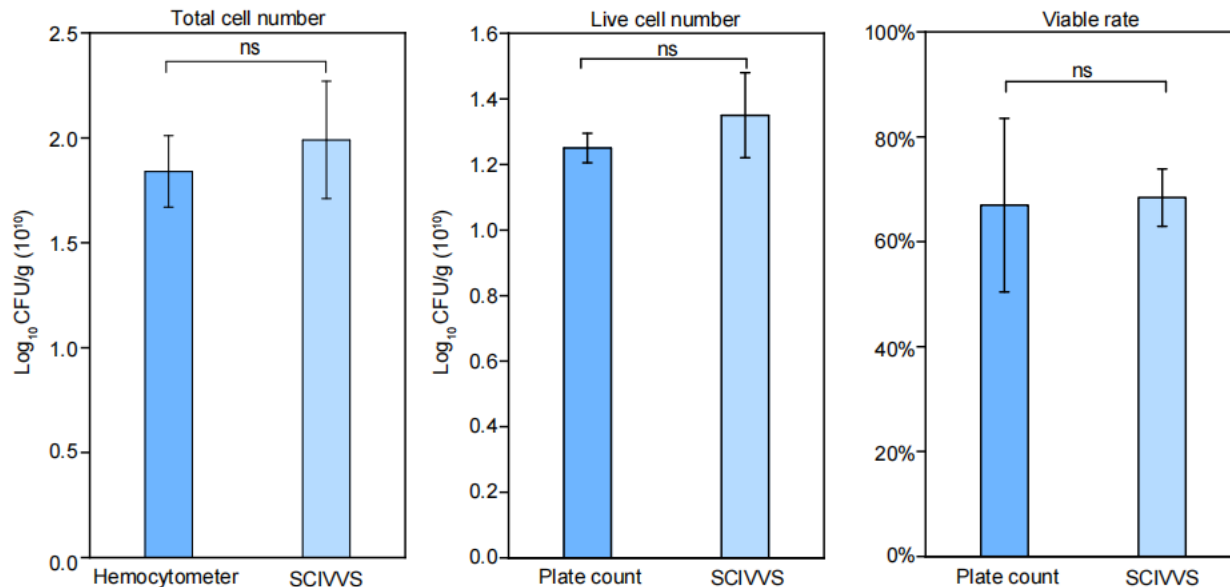
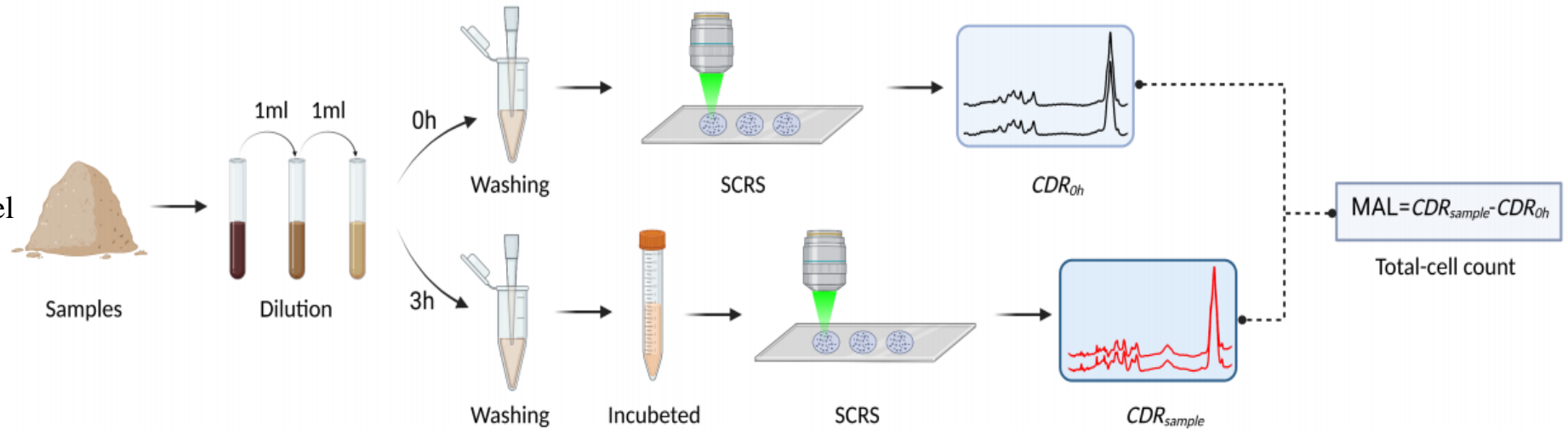
Count the total-cell count, live-cell count, and viable rate of MPP-A based on the traditional method or SCIVVS

$MAL \leq 0$, dead cells

$MAL > 0$, live cells

rMAL: relative Metabolic Activity Level

$$rMAL = \frac{CDR_{sample} - CDR_{0h}}{CDR_{control} - CDR_{0h}}$$



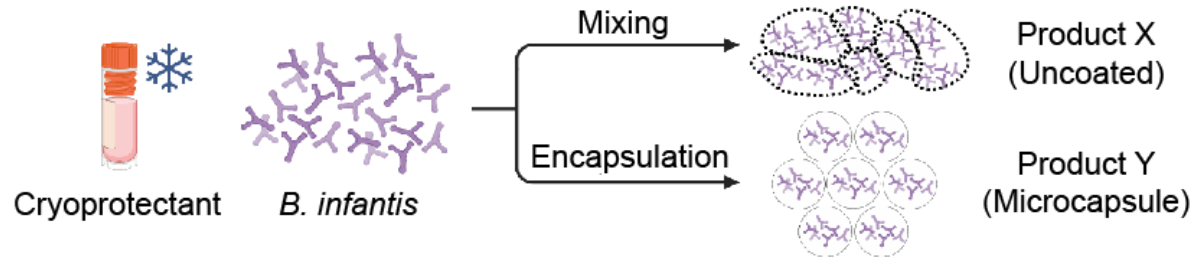
The index of Renhe single probiotic product (MPP-A)

- SCIVVS can perform accurate the total-cell count, live-cell count, and viable rate of probiotic products
- No significant difference compared to the traditional method

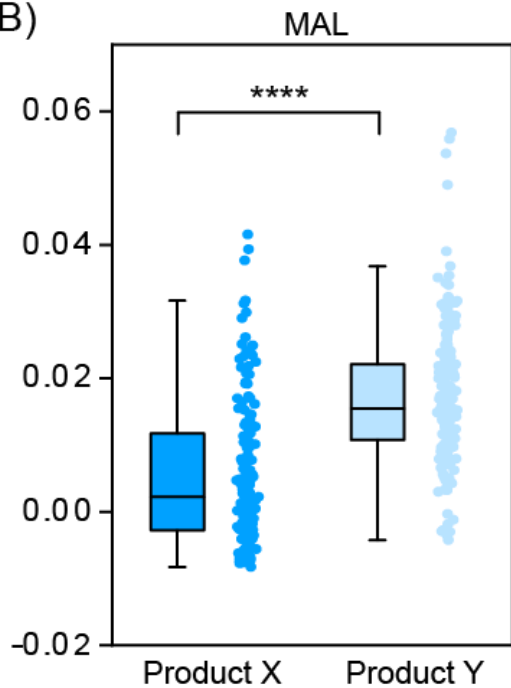


SCRS-based in situ vitality test evaluate the different processing technologies

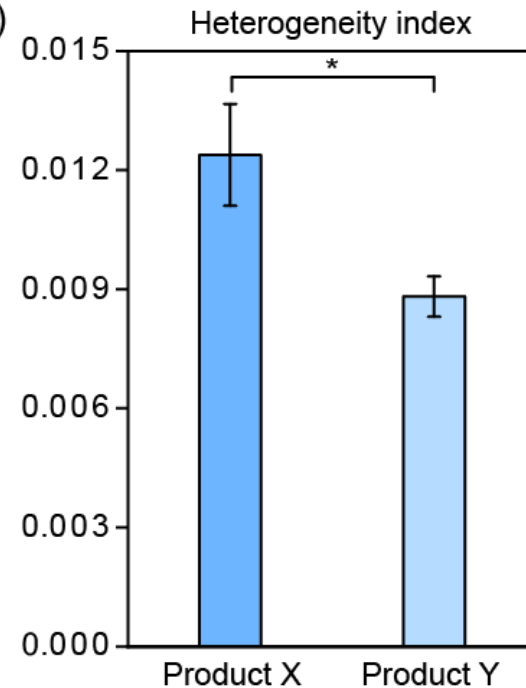
(A)



(B)



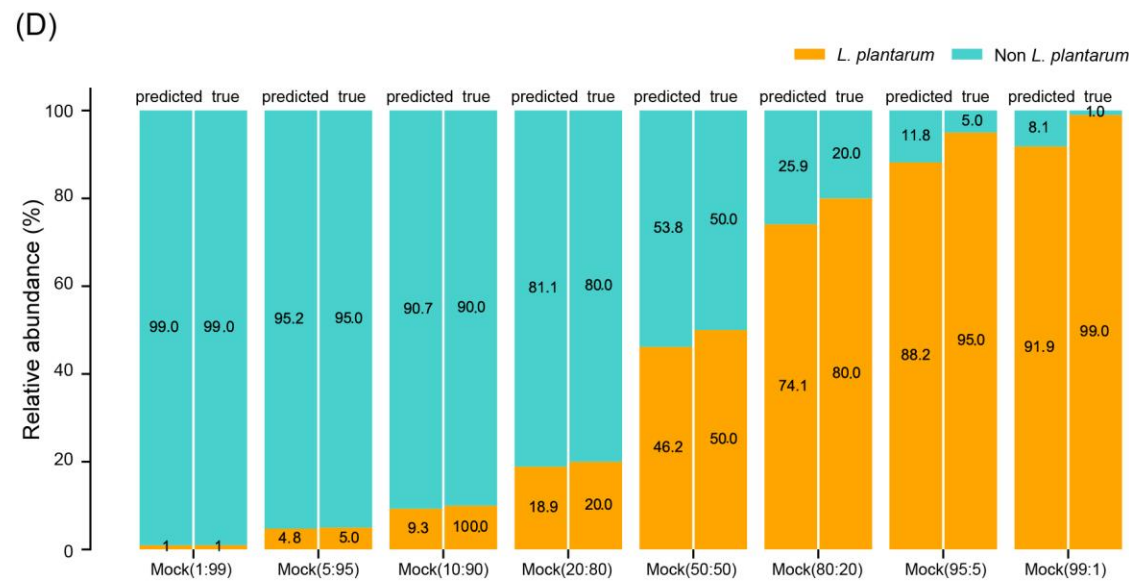
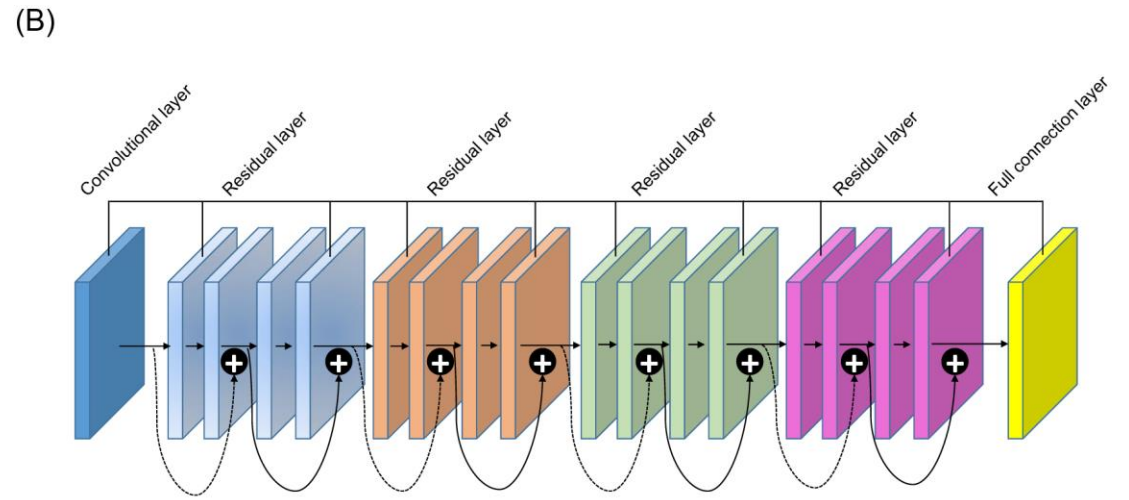
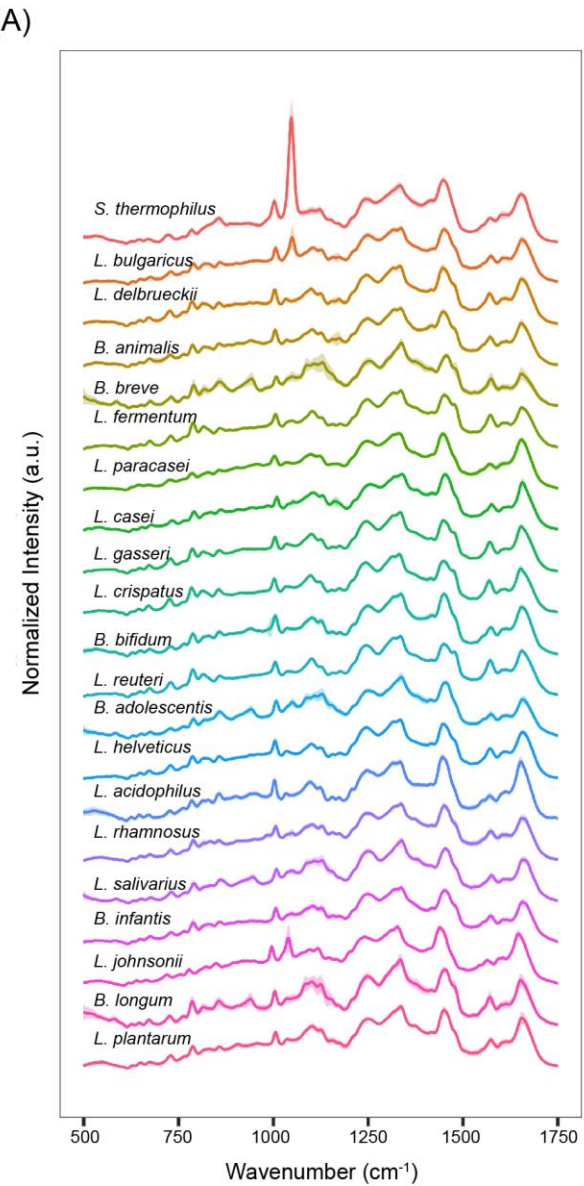
(C)



- Culture-based live-cell counts, the viability is equivalent
- The acceleration experiment is time-consuming **(10 days)**
- SCIVVS takes just **5 h** can quantitatively distinguish the processing technologies by the MAL and HI
- Higher cellular vitality and more homogeneous for Y than X
- **Therefore, the microencapsulation coating helps to maintain the metabolic viability of probiotics**



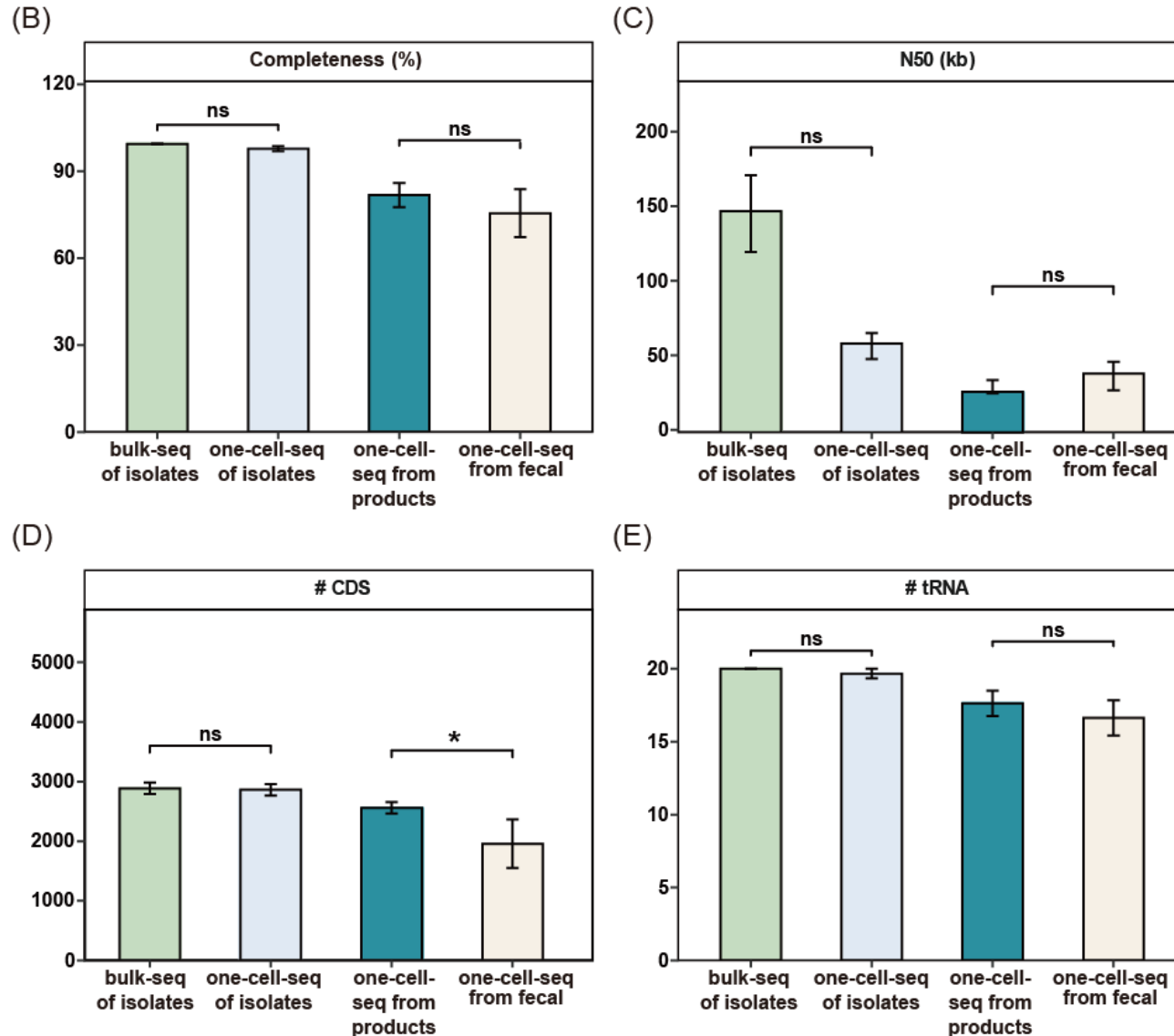
SCRS-based ID of probiotic cells from commercial probiotic products



- Build a reference database of SCRS from 21 standard probiotic strains
- The SCRS-based CNN model reports an identification accuracy of **93%** (directly from actual probiotic products)
- The mock community, distinguish *L. plantarum* 299V single cells from other probiotic cells (with discrepancy between predicted and actual results <3.5%)



Source-tracking of single cells in probiotic products via one-cell genome sequences using scRACS-Seq in SCIVVS

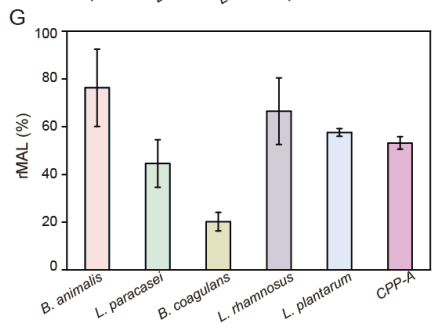
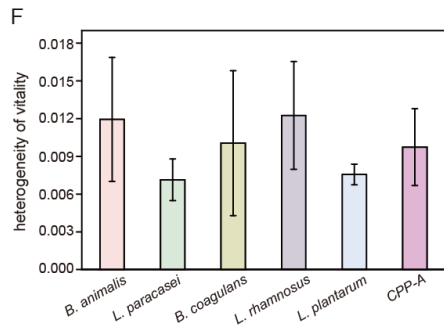
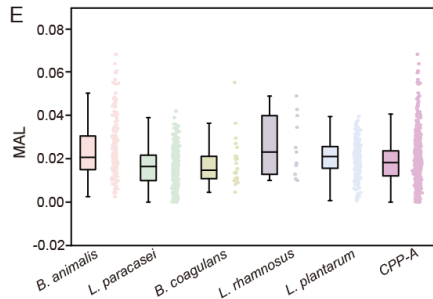
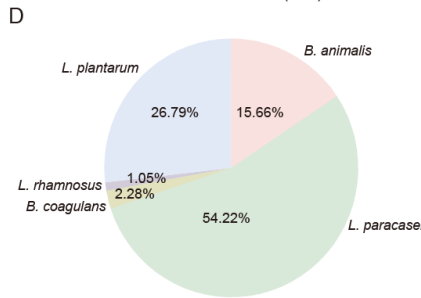
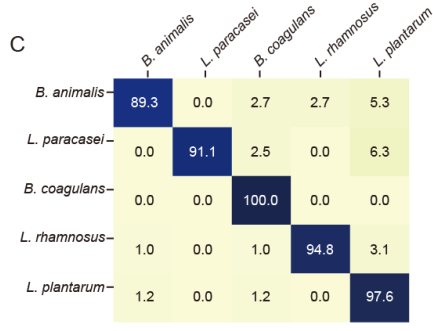
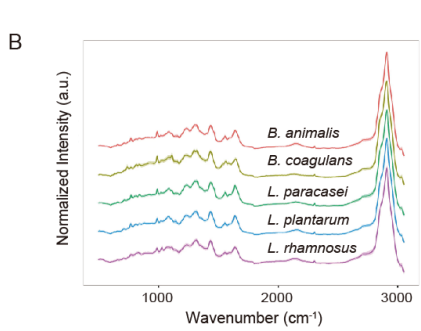
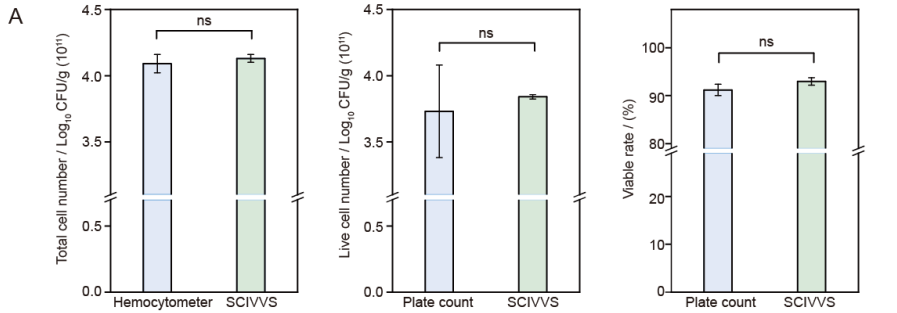


Probiotics pure strain or compound probiotic product (*L. paracasei*, *L. rhamnosus*, *L. plantarum*)

- Single cell genome completeness at $99.40 \pm 0.05\%$
- Single-cell sequencing of all pure strains (>95% coverage) with comparable quality to bulk
- the average completeness is 81.79% , no significant difference with fecal (SAG-gel platform)
- **Therefore, SCIVVS can reliably source-tracking strains directly from probiotic products at single-cell resolution**



An integrated single-cell SCIVVS workflow for quality assessment of compound probiotic products



The live-cell counts and proportions of each strain in CPP-A

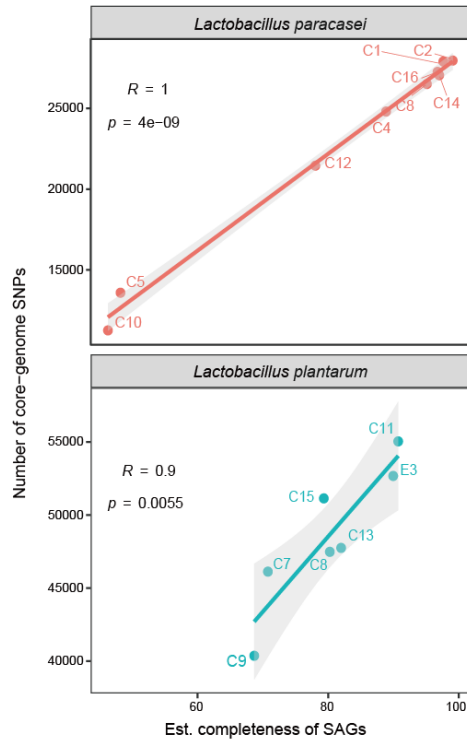
Strains	Live cells proportion		Live cells number SCIVVS
	Actual proportion	SCIVVS	
<i>B. animalis</i>	17.08%	15.66 ± 0.01%	6.01 ± 0.05 × 10 ¹⁰ CFU/g
<i>L. paracasei</i>	54.10%	54.22 ± 0.03%	2.08 ± 0.10 × 10 ¹¹ CFU/g
<i>B. coagulans</i>	2.02%	2.28 ± 0.01%	8.76 ± 0.03 × 10 ⁹ CFU/g
<i>L. rhamnosus</i>	0.56%	1.05 ± 0.01%	4.03 ± 0.02 × 10 ⁹ CFU/g
<i>L. plantarum</i>	26.24%	26.79 ± 0.01%	1.03 ± 0.04 × 10 ¹¹ CFU/g

- Build a reference SCRS database of five strains
- The average accuracy of SCRS-based ID is **94.93 ± 0.01%**
- The total-cell count, live-cell count, and viable rate of CPP-A (compound probiotic product) based on SCIVVS with no significant difference compared to the traditional method
- SCRS-based ID for the five strains in CPP-A is accurate
- The number of viable cells, MAL, rMAL, and HI of each strain in the product

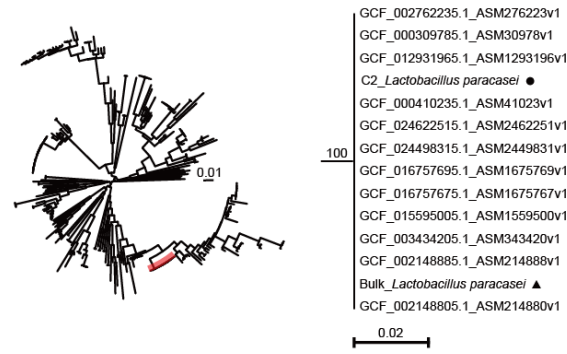


Source-tracking of single cells in multistrain probiotic products

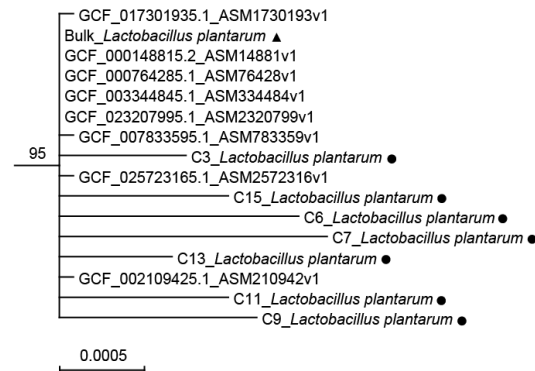
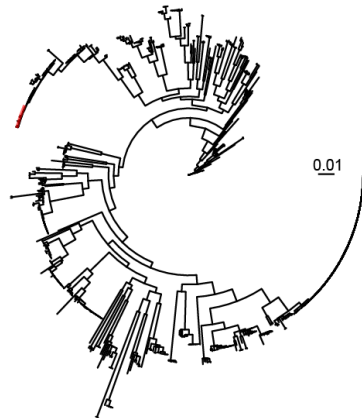
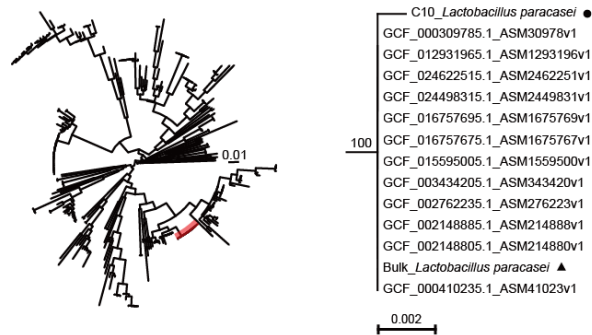
(H)



(I)

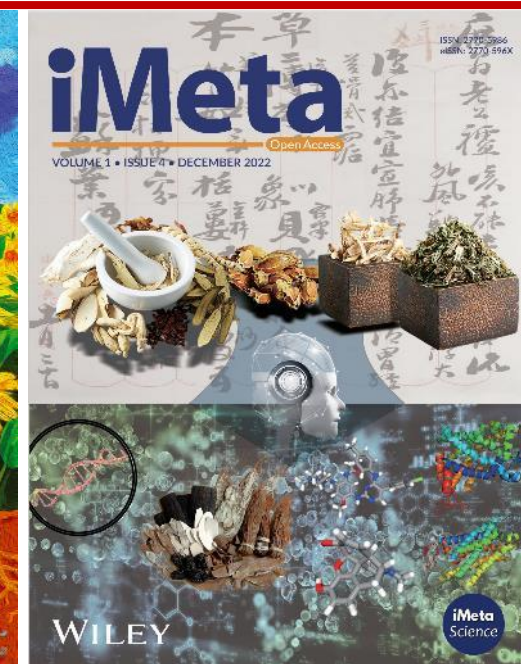


(J)



- The number of core-genome SNPs is positively correlated with the completeness of SAGs
- *L. paracasei*: the lowest-coverage SAG (C10, 46.28%), the highest-coverage SAG (C2, 99.07%), both can accurately source-track
- *L. plantarum*: at the one-cell genome coverage of 68.68%–90.72%, accurate source-tracking results were also obtained
- Therefore, SCIVVS can source-track the of probiotic products at the one-cell level, regardless of the cultivability, are sufficient to support sensitive and reliable source-tracking





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