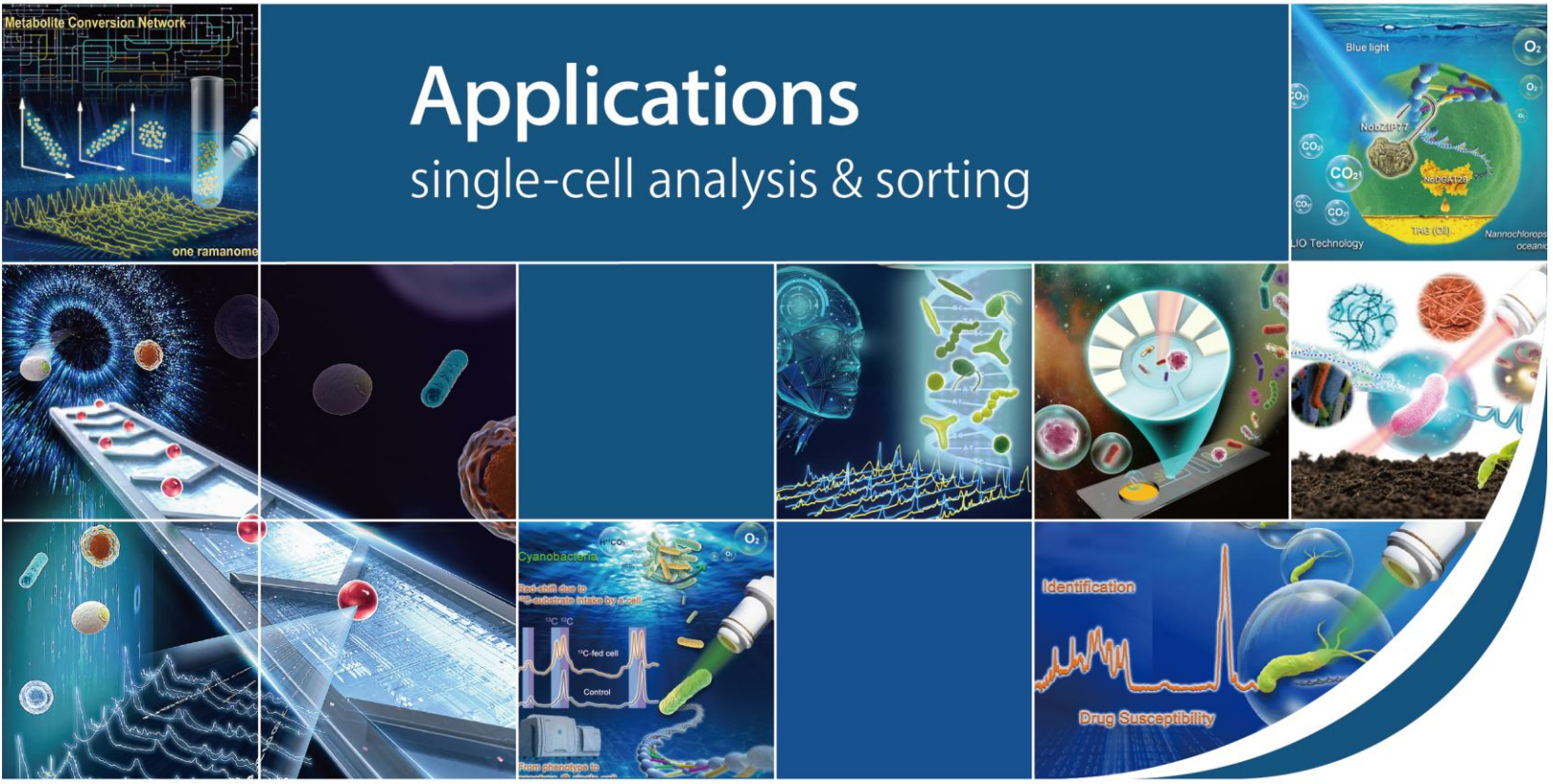
Applications

single-cell analysis & sorting



Contents

PNAS RESEARCH ARTICLE | APPLIED BIOLOGICAL SCIENCES

Label-free high-throughput live-cell sorting of genome-wide random mutagenesis libraries for metabolic traits by Raman flow cytometry

Xixian Wang[a,b,c,d,1], Sen Wang[a,b,c,d,e,1], Zhidian Diao[a,b,c,d,1], Xibao Hou[f,1], Yanhai Gong[a,b,c,d], Qing Sun[a,b,c,d], Jiaping Zhang[a,b,c], Lihui Ren[a,b,c,d], Yuandong Li[a,b,c,d], Yuetong Ji[f], Wei Shen[a,b,c,d], Yifeng Yin[g], Shi Huang[h], Xiaojin Song[a,b,c,d,e], Qiu Cui[a,b,c,d,e], Yingang Feng[a,b,c,d,e,2], Jian Xu[a,b,c,d,2], and Bo Ma[a,b,c,d,2]

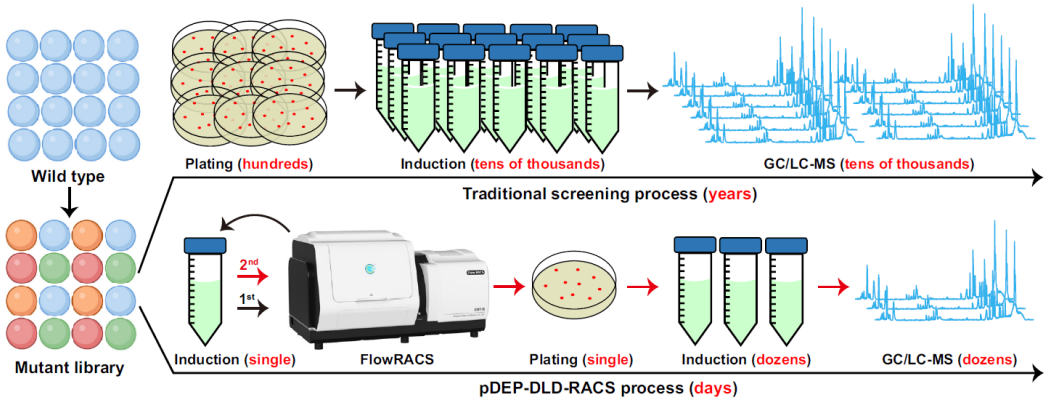
Abstract

A full spontaneous single-cell Raman spectrum captures the metabolic phenome in a label-free and noninvasive manner. However, Raman-activated cell sorting (RACS) of rare target cells from highly heterogeneous systems has remained largely conceptual.

Here, we present a positive dielectrophoresis-induced deterministic lateral displacement (pDEP-DLD)- based RACS (pDEP-DLD- RACS), in which a modulated pDEP-DLD force is applied to focus, trap, and functionally sort fast-moving single cells in a wide channel. For pigment-and oil-producing yeasts, pDEP-DLD- RACS shows high sorting accuracy (>90%), high throughput (~600 events min^{-1}), high yield (>85%), and long stable running time (~10 h), and can sort rare cells while preserving full cellular vitality.

Moreover, label-free sorting directly from a genome-wide random mutagenesis library with >10^5 *Aurantiochytrium* sp. mutants, based on intracellular docosahexaenoic acid (DHA) content, produces mutant cells with 58% higher DHA productivity in just two RACS runs over two days, representing two-orders- of-magnitude higher time-and cost-efficiency than conventional approaches.

This superior trait arises from global remolding of transcriptomes, including enhanced carbon metabolism, reduced intracellular NADPH synthesis rates, and increased triacylglycerol (TAG) synthesis. By enabling direct screening of metabolic traits from genome-wide mutagenesis libraries, pDEP-DLD- RACS is a powerful platform for synthetic biology.



Highlight

- FlowRACS enables high-throughput, noninvasive screening of DHA-hyperproducing single cells through a screen-first-and-culture-next approach from a genome-wide random mutagenesis library of *Aurantiochytrium* sp., significantly streamlining the process compared to conventional strategies

- Wang X, *et al*. Label-free high-throughput live-cell sorting of genome-wide random mutagenesis libraries for metabolic traits by Raman flow cytometry. *Proc Natl Acad Sci U S A*. 2025, 122(22): e2503641122. https://doi.org/10.1073/pnas.2503641122.

ACS SyntheticBiology

pubs.acs.org/synthbio Research Article

Iron-Derepressed Robust Production of Fusarinine C Siderophore by *Aureobasidium melanogenum*

Xinxin Kang,[#] Weixing Liu,[#] Xixian Wang, Muhammad Aslam, Guanglei Liu,[*] and Zhe Chi[*]

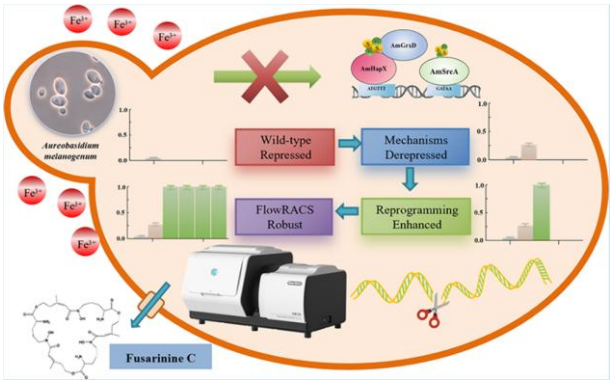
Abstract

Siderophore biosynthesis is severely inhibited by iron sufficiency, limiting microbial production of siderophores on a large industrial scale.

Herein, we report novel iron-derepressed and robust production of the fungal siderophore fusarinine C (FsC) in *Aureobasidium melanogenum*, achieved by metabolic reprogramming coupling Raman-based single-cell sorting (RACS). First, we deciphered the mechanisms of iron repression on siderophore biosynthesis in this fungus. Guided by this, we constructed an iron-depressed chassis. In this chassis, we reprogrammed the metabolic pathways of FsC involving the modules of L-ornithine, mevalonate, and siderophore biosynthesis, accomplishing iron-depressed, robust, and sole production of FsC with an extracellular titer of 763 mg/L under iron-sufficient conditions in stainless steel fermenters. However, intrinsic heterogenicity occurred in FsC bioproduction.

To overcome this, we developed an approach based on flow-mode RACS to select and acquire high FsC-producing single cells from each fermentation batch to be used as the seed for the next batch, thereby persistently maintaining the robustness of the FsC titer over 818 mg/L in successive batch fermentation.

This study showcases the principle for overcoming iron repression and heterogenicity of siderophore bioproduction to fit large-scale industrialization.



Highlight

- FlowRACS precisely screens and enriches high-FsC-producing single cells to be used as seed culture for each fermentation batch, and the FsC yield remains stable across four consecutive batches. In contrast, the control group without FlowRACS screening shows a gradual yield decline.

- Kang X, *et al*. Iron-Derepressed Robust Production of Fusarinine C Siderophore by *Aureobasidium melanogenum*. *ACS Synth Biol*. 2025, 14(5):1625-1637. https://doi.org/10.1021/acssynbio.4c00897.

analytical chemistry
Article
Cite This: Anal. Chem. 2017, 89, 12569-12577
pubs.acs.org/ac

Raman-Activated Droplet Sorting (RADS) for Label-Free High-Throughput Screening of Microalgal Single-Cells

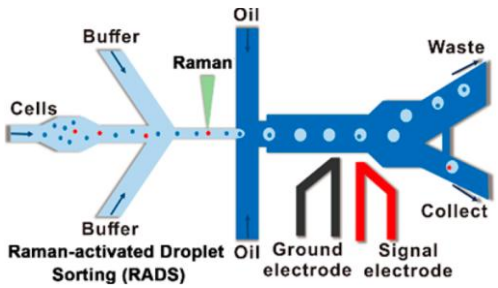
Xixian Wang,[†,§,⊥] Lihui Ren,[†,§,⊥] Yetian Su,[†] Yuetong Ji,[†,§] Yaoping Liu,[∥] Chunyu Li,[†,§] Xunrong Li,[†,§] Yi Zhang,[†] Wei Wang,[∥] Qiang Hu,[‡] Danxiang Han,[‡] Jian Xu,[*,†,§] and Bo Ma[*,†,§]

Abstract

Raman-activated cell sorting (RACS) has attracted increasing interest, yet throughput remains one major factor limiting its broader application.

Here we present an integrated Raman-activated droplet sorting (RADS) microfluidic system for functional screening of live cells in a label-free and high-throughput manner, by employing AXT-synthetic industrial microalga *Haematococcus pluvialis* (*H. pluvialis*) as a model. Raman microspectroscopy analysis of individual cells is carried out prior to their microdroplet encapsulation, which is then directly coupled to DEP-based droplet sorting. To validate the system, *H. pluvialis* cells containing different levels of AXT were mixed and underwent RADS. Those AXT-hyperproducing cells were sorted with an accuracy of 98.3%, an enrichment ratio of eight folds, and a throughput of ~260 cells/min. Of the RADS-sorted cells, 92.7% remained alive and able to proliferate, which is equivalent to the unsorted cells.

Thus, the RADS achieves a much higher throughput than existing RACS systems, preserves the vitality of cells, and facilitates seamless coupling with downstream manipulations such as single-cell sequencing and cultivation.



Highlight

- Raman-activated droplet sorting (RADS) enables high-throughput, label-free functional cell sorting with high accuracy while preserving cell viability for downstream analysis.

- Wang X, *et al*. Raman-Activated Droplet Sorting (RADS) for Label-Free High-Throughput Screening of Microalgal Single-Cells. Anal Chem. 2017, 89(22): 12569-12577.
https://doi.org/10.1021/acs.analchem.7b03884

analytical chemistry
Article
pubs.acs.org/ac

Raman-Activated Cell Sorting Based on Dielectrophoretic Single-Cell Trap and Release

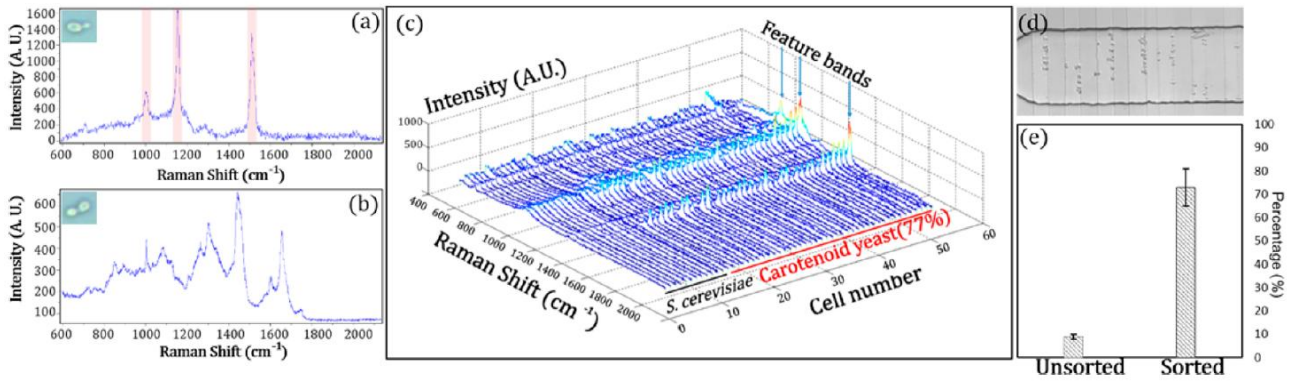
Peiran Zhang,[†,‡] Lihui Ren,[†] Xu Zhang,[†] Yufei Shan,[†] Yun Wang,[†] Yuetong Ji,[†] Huabing Yin,[∥] Wei E. Huang,[†,§] Jian Xu,*[,†] and Bo Ma*[,†]

Abstract

Raman-activated cell sorting (RACS) is a promising single-cell technology that holds several significant advantages, as RACS is label-free, information-rich, and potentially in situ. To date, the ability of the technique to identify single cells in a high-speed flow has been limited by inherent weakness of the spontaneous Raman signal.

Here we present an alternative pause-and-sort RACS microfluidic system that combines positive dielectrophoresis (pDEP) for single-cell trap and release with a solenoid-valve-suction-based switch for cell separation. This has allowed the integration of trapping, Raman identification, and automatic separation of individual cells in a high-speed flow. By exerting a periodical pDEP field, single cells were trapped, ordered, and positioned individually to the detection point for Raman measurement.

As a proof-of-concept demonstration, a mixture of two cell strains containing carotenoid-producing yeast (9%) and non-carotenoid-producing *Saccharomyces cerevisiae* (91%) was sorted, which enriched the former to 73% on average and showed a fast Raman-activated cell sorting at the subsecond level.



Highlight

- FlowRACS sorting efficiency was validated by sorting a mixture of the carotenoid-producing yeast (9% of the population) and the non-carotenoid producing *S. cerevisiae* (91% of the population). Raman spectra of these two cell types showed significant differences with distinct Raman bands at 1105, 1155, and 1510 cm^{-1} (highlighted with red squares. The difference were not observable through single-cell microscopy.

- Zhang P, *et al*. Raman-activated cell sorting based on dielectrophoretic single-cell trap and release. *Anal Chem*. 2015, 87(4): 2282-9. https://doi.org/10.1021/ac503974e

SCIENCE ADVANCES | RESEARCH ARTICLE

MICROBIOLOGY

Positive dielectrophoresis–based Raman-activated droplet sorting for culture-free and label-free screening of enzyme function in vivo

Xixian Wang[1,2,3]*, Yi Xin[1,2,3]*, Lihui Ren[1,2,3]*, Zheng Sun[1,2,3], Pengfei Zhu[1,2,3], Yuetong Ji[1,2,3,4], Chunyu Li[1,2,3], Jian Xu[1,2,3†], Bo Ma[1,2,3†]

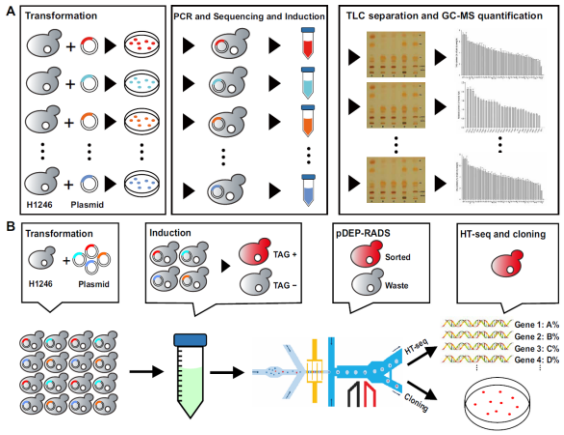
Abstract

The potential of Raman-activated cell sorting (RACS) is inherently limited by conflicting demands for signal quality and sorting throughput.

Here, we present positive dielectrophoresis-based Raman-activated droplet sorting (pDEP-RADS), where a periodical pDEP force was exerted to trap fast-moving cells, followed by simultaneous microdroplet encapsulation and sorting. Screening of yeasts for triacylglycerol (TAG) content demonstrated near-theoretical-limit accuracy, ~120 cells min^{-1} throughput and full-vitality preservation, while sorting fatty acid degree of unsaturation (FA-DU) featured ~82% accuracy at ~40 cells min^{-1}.

From a yeast library expressing algal diacylglycerol acyltransferases (DGATs), a pDEP-RADS run revealed all reported TAG-synthetic variants and distinguished FA-DUs of enzyme products. Furthermore, two previously unknown DGATs producing low levels of monounsaturated fatty acid-rich TAG were discovered.

This first demonstration of RACS for enzyme discovery represents hundred-fold saving in time consumables and labor versus culture-based approaches. The ability to automatically flow-sort resonance Raman-independent phenotypes greatly expands RACS' application.



Highlight

- FlowRACS enables rapid, high-throughput screening of *in vivo* DGAT gene functions through parallel gene transformation and functional sorting, streamlining the workflow by eliminating time-consuming traditional experimental steps, including clone isolation, cultivation, and enzyme product analysis.

- Wang X, *et al*. Positive dielectrophoresis-based Raman-activated droplet sorting for culture-free and label-free screening of enzyme function in vivo. *Sci Adv*. 2020, 6(32): eabb3521. https://doi.org/10.1126/sciadv.abb3521

RESEARCH ARTICLE

iMetaOmics WILEY

Raman flow cytometry based single-cell species classification, viable-cell counting and vitality test for probiotic products

Jia Zhang[1,2,3] | Jianmei Wang[1,3] | Pengfei Zhu[1,2,4,5] | Zhidian Diao[1,3] | Shuhua Tian[4] | Ziyuan Ding[6] | Yongming Duan[6] | Teng Xu[1] | Xuan Zhou[1] | Xixian Wang[1,2,3] | Xia Ma[7,8] | Ting Sun[7,8] | Xiaoyan Jing[1,2,3] | Weilian Hung[7,8] | Bo Ma[1,2,3] | Shi Huang[9] | Xiaowei Zheng[6] | Jian Xu[1,2,3]

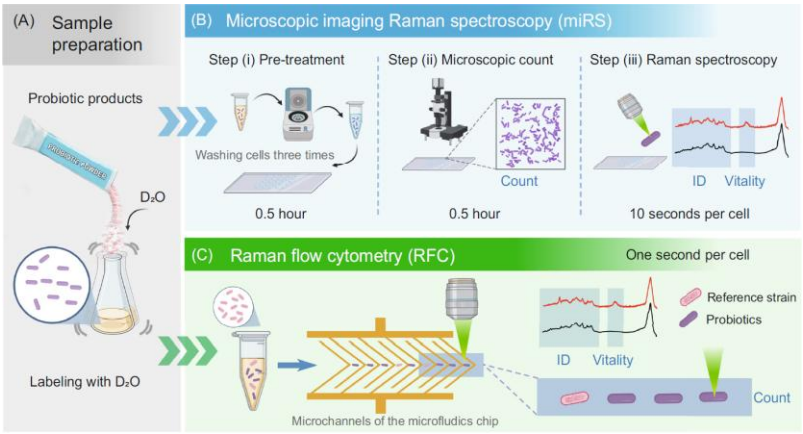
Abstract

The rapidly expanding probiotic industry demands efficient methods for quality assessment of probiotic products. We previously established a microscopic imaging and Raman spectroscopy (miRS) based approach for rapid identification and tests for viability and vitality at single-cell resolution via single-cell Raman spectra (SCRS), yet its wider application is limited by the throughput of SCRS acquisition.

Here, we employed a Raman flow cytometry (RFC) approach to accomplish this mission, which features a greatly simplified experimental workflow yet with >10-fold higher throughput than miRS. At SCRS acquisition speed of one bacterial cell per second, RFC achieves much higher sampling depth, leading to higher accuracy of species classification than miRS in both reference ramanome database construction and quality assessment of probiotic products.

Specifically, based on the fingerprint regions, the accuracy in classifying species and strains is notably improved, both for an isogenic population and for a multi-strain probiotic product. Moreover, for probiotic products with highly biased compositions, the sensitivity of detecting and classifying low-abundance members is order-of-magnitude higher. Furthermore, based on the fingerprint regions plus the C-D band, D_2O-probed RFC rapidly yields viable-cell counts and quantifies single-cell vitality in a species/strain-resolved fashion yet without the need for fluorescence labeling, underscoring its strength over propidium monoazide (PMA)-based fluorescence flow cytometry.

Due to its speed, accuracy, sensitivity, rich information and ease of use, RFC is a powerful platform for quality assessment for probiotics and other live-cell products.



Highlight

- FlowRACS enables total cell counting, rapid identification, and strain-level *in situ* viability/vitality analysis of commercial probiotic products.

- Zhang J, *et al*. Raman flow cytometry based single-cell species classification, viable-cell counting and vitality test for probiotic products. iMetaOmics, 2025: e70024.
https://doi.org/10.1002/imo2.70024

RESEARCH ARTICLE

iMeta WILEY

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

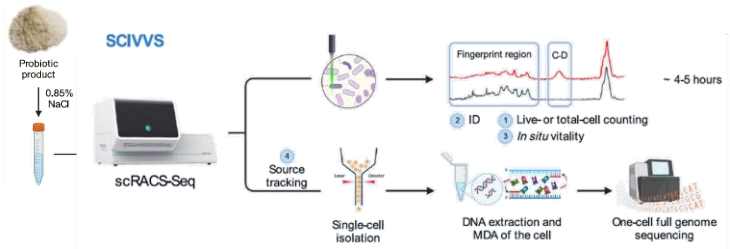
Jia Zhang[1,2,3,4] | Lihui Ren[1,2,3,5] | Lei Zhang[1,2,3,6] | Yanhai Gong[1,2,3,4] | Teng Xu[1,2,3,4] | Xiaohang Wang[1,2,3,4] | Cheng Guo[7] | Lei Zhai[8] | Xuejian Yu[8] | Ying Li[9] | Pengfei Zhu[1,9] | Rongze Chen[1,2,3,4] | Xiaoyan Jing[1,2,3,4] | Gongchao Jing[1,2,3,4] | Shiqi Zhou[1,2,3] | Mingyue Xu[1,2,3] | Chen Wang[1,2,3] | Changkai Niu[7] | Yuanyuan Ge[8] | Bo Ma[1,2,3,4] | Gaishuang Shang[7] | Yunlong Cui[7] | Su Yao[8] | Jian Xu[1,2,3,4]

Abstract

Rapid expansion of the probiotics industry demands fast, sensitive, comprehensive, and low-cost strategies for quality assessment.

Here, we introduce a culture-free, one-cell-resolution, phenome-genome-combined strategy called Single-Cell Identification, Viability and Vitality tests, and Source-tracking (SCIVVS). For each cell directly extracted from the product, the fingerprint region of D_2O-probed single-cell Raman spectrum (SCRS) enables species level identification with 93% accuracy, based on a reference SCRS database from 21 statutory probiotic species, whereas the C–D band accurately quantifies viability, metabolic vitality plus their intercellular heterogeneity. For source-tracking, single-cell Raman-activated Cell Sorting and Sequencing can proceed, producing indexed, precisely one-cell-based genome assemblies that can reach ~99.40% genome-wide coverage.

Finally, we validated an integrated SCIVVS workflow with automated SCRS acquisition where the whole process except sequencing takes just 5 h. As it is >20-fold faster, >10-time cheaper, vitality-revealing, heterogeneity-resolving, and automation-prone, SCIVVS is a new technological and data framework for quality assessment of live-cell products.



Highlight

- RACS-Seq enables total viable cell counting, rapid microbial species identification, species-resolved *in situ* vitality assessment, and microbial source tracking at single-cell resolution directly from commercial probiotic products.

- Zhang J, *et al*. Single-cell rapid identification, in situ viability and vitality profiling, and genome-based source-tracking for probiotics products. *iMeta*. 2023, 2(3): e117. https://doi.org/10.1002/imt2.117.

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

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Original Research Article

Culture-free identification of fast-growing cyanobacteria cells by Raman-activated gravity-driven encapsulation and sequencing

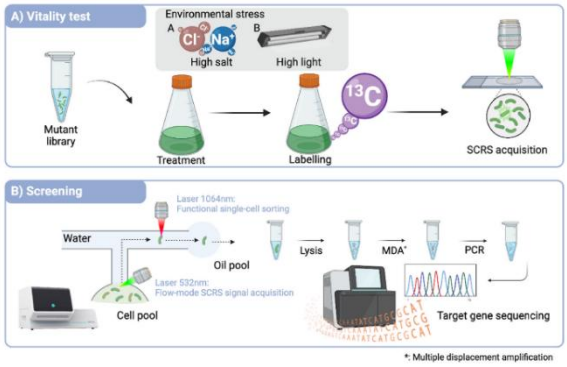
Jinyu Cui [a,b,c,1], Rongze Chen [a,b,c,d,1], Huili Sun [a,b,c,d,1], Yingyi Xue [a,b,c], Zhidian Diao [a,b,c,d], Jingyun Song [a,b,c], Xiaohang Wang [a,b,c], Jia Zhang [a,b,c], Chen Wang [a,b,c], Bo Ma [a,b,c], Jian Xu [a,b,c,d,f,**], Guodong Luan [a,b,c,d,e,***], Xuefeng Lu [a,b,c,d,e,f,*]

Abstract

By directly converting solar energy and carbon dioxide into biobased products, cyanobacteria are promising chassis for photosynthetic biosynthesis. To make cyanobacterial photosynthetic biosynthesis technology economically feasible on industrial scales, exploring and engineering cyanobacterial chassis and cell factories with fast growth rates and carbon fixation activities facing environmental stresses are of great significance.

To simplify and accelerate the screening for fast-growing cyanobacteria strains, a method called Individual Cyanobacteria Vitality Tests and Screening (iCyanVS) was established. We show that the ^{13}C incorporation ratio of carotenoids can be used to measure differences in cell growth and carbon fixation rates in individual cyanobacterial cells of distinct genotypes that differ in growth rates in bulk cultivations, thus greatly accelerating the process screening for fastest-growing cells. The feasibility of this approach is further demonstrated by phenotypically and then genotypically identifying individual cyanobacterial cells with higher salt tolerance from an artificial mutant library via Raman-activated gravity-driven encapsulation and sequencing.

Therefore, this method should find broad applications in growth rate or carbon intake rate based screening of cyanobacteria and other photosynthetic cell factories.



Highlight

- RACS-Seq combines single-cell phenotyping and genotyping by first identifying stress-tolerant mutants through ^{13}C incorporation, followed by automatically sorting and sequencing them in a single integrated workflow.

- Cui J, *et al*. Culture-free identification of fast-growing cyanobacteria cells by Raman-activated gravity-driven encapsulation and sequencing. *Synth Syst Biotechnol*. 2023, 8(4): 708-715. https://doi.org/10.1016/j.synbio.2023.11.001

frontiers | Frontiers in Microbiology

Rapid identification of lactic acid bacteria at species/subspecies level via ensemble learning of Ramanomes

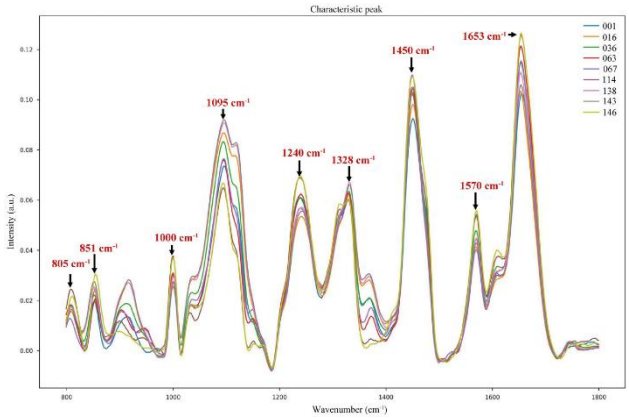
Yan Ren[1,2], Yang Zheng[1], Xiaojing Wang[1], Shuang Qu[1], Lijun Sun[3], Chenyong Song[4], Jia Ding[4], Yuetong Ji[3,4], Guoze Wang[1,2], Pengfei Zhu[3,4]* and Likun Cheng[1,2]*

Abstract

Rapid and accurate identification of lactic acid bacteria (LAB) species would greatly improve the screening rate for functional LAB. Although many conventional and molecular methods have proven efficient and reliable, LAB identification using these methods has generally been slow and tedious.

Single-cell Raman spectroscopy (SCRS) provides the phenotypic profile of a single cell and can be performed by Raman spectroscopy (which directly detects vibrations of chemical bonds through inelastic scattering by a laser light) using an individual live cell. Recently, owing to its affordability, non-invasiveness, and label-free features, the Ramanome has emerged as a potential technique for fast bacterial detection. Here, we established a reference Ramanome database consisting of SCRS data from 1,650 cells from nine LAB species/subspecies and conducted further analysis using machine learning approaches, which have high efficiency and accuracy. We chose the ensemble meta-classifier (EMC), which is suitable for solving multi-classification problems, to perform in-depth mining and analysis of the Ramanome data. To optimize the accuracy and efficiency of the machine learning algorithm, we compared nine classifiers: LDA, SVM, RF, XGBoost, KNN, PLS-DA, CNN, LSTM, and EMC. EMC achieved the highest average prediction accuracy of 97.3% for recognizing LAB at the species/subspecies level.

In summary, Ramanomes, with the integration of EMC, have promising potential for fast LAB species/subspecies identification in laboratories and may thus be further developed and sharpened for the direct identification and prediction of LAB species from fermented food.



Highlight

- FlowRACS enables rapid identification of lactic acid bacteria (LAB) species and subspecies by detecting characteristic peaks in the fingerprint region using Raman spectroscopy and ensemble learning algorithms.

- Ren Y, *et al*. Rapid identification of lactic acid bacteria at species/subspecies level via ensemble learning of Ramanomes. *Frontiers in Microbiology*. 2024, 15: 1361180.
https://doi.org/10.3389/fmicb.2024.1361180

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

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Original Research Article

Assessing the physiological properties of baker's yeast based on single-cell Raman spectrum technology

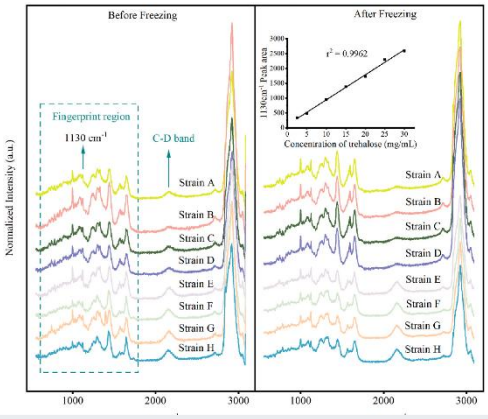
Xi Sun [a,c,**,1], Xin Zhou [a,1], Ran Yu [f], Xiaofang Zhou [f], Jun Zhang [a], Teng Xu [b], Jianmei Wang [b], Mengqi Li [a], Xiaoting Li [a], Min Zhang [a], Jian Xu [b,d,e], Jia Zhang [b,d,e,*]

Abstract

With rapid progress in the yeast fermentation industry, a comprehensive commercial yeast quality assessment approach integrating efficiency, accuracy, sensitivity, and cost-effectiveness is required.

In this study, a new yeast quality assessment method based on single-cell Raman technology was developed and contrasted with traditional methods. The findings demonstrated significant associations (Pearson correlation coefficient of 0.933 on average) between the two methods in measuring physiological indicators, including cell viability and intracellular trehalose content, demonstrating the credibility of the Raman method compared to the traditional method. Furthermore, the sensitivity of the Raman method in viable but non-culturable cells was higher in measuring yeast cell viability (17.9 % more sensitive). According to the accurate quantitative analysis of metabolic activity level (MAL) of yeast cells, the cell vitality was accurately quantified at population and single-cell levels, offering a more comprehensive assessment of yeast fermentation performance.

Overall, the single-cell Raman method integrates credibility, feasibility, accuracy, and sensitivity in yeast quality assessment, offering a new technological framework for quality assessments of live-cell yeast products.



Highlight

- FlowRACS enables rapid, low-cost, all-in-one quality assessment of baker's yeast via single-cell Raman spectroscopy, simultaneously evaluating metabolic activity and freezing resistance.

- Sun X, *et al*. Assessing the physiological properties of baker's yeast based on single-cell Raman spectrum technology. *Synthetic and Systems Biotechnology*. 2025, 10(1): 110-118. https://doi.org/10.1016/j.synbio.2024.09.004

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Optical-based microbubble for on-demand droplet release from static droplet array (SDA) for dispensing one droplet into one tube

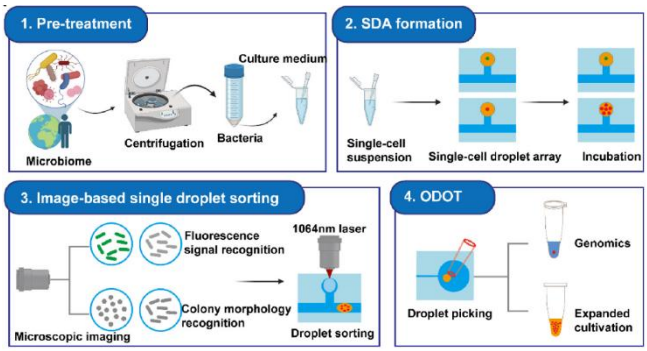
Zhidian Diao [a,b,c,d,1], Xixian Wang [a,b,c,d,1], Jiaping Zhang [a,c,d], Anle Ge [a,c,d], Teng Xu [a,b,c,d], Lingyan Kan [a,c,d], Yuandong Li [a,c,d], Yuetong Ji [a,e], Xiaoyan Jing [a,b,c,d], Jian Xu [a,b,c,d,**], Bo Ma [a,b,c,d,*]

Abstract

Static droplet array (SDA) is a pivotal tool for high-capacity screening assays, yet extraction and collection the target droplets that contain unique analytes or cells from the SDA remains one major technical bottleneck that limits its broader application.

Here we present an optical-based on-demand droplet release (OODR) system by incorporating a 1064 nm laser-responsive indium tin oxide (ITO) layer into a chamber array-based droplet microfluidic chip. By focusing the 1064 nm laser onto the ITO layer, microbubbles can be created via local heating to selectively push-out the droplets from the chamber. Then the released droplet is readily exported in a one-droplet-one-tube (ODOT) manner by the inherent capillary force into pipette tip. Releasing of the droplets containing fluorescein sodium demonstrated ~100% successful rate (9 out of 6,400 droplets were successfully released) and low residual (only ~5% of the droplet volume remains in the chamber). White or fluorescence image-based releasing of single-cell-droplets directly after cell loading or multi-cells-droplets derived from on chip single-cell cultivation for both *E. coli* and yeast cells further demonstrated the wide applicability of OODR.

The present system is user-friendly and has the potential to be applied in various high-throughput screening assays, including single molecule/cell analysis, drug screening, and phenotype-based cell sorting.



Highlight

- DCP utilizes an optical-based microbubble technology to enable precise target clone recovery with ~ 100% efficiency and less than 5% residual volume, facilitating high-throughput single-cell cultivation and screening applications.

- Diao Z, *et al*. Optical-based microbubble for on-demand droplet release from static droplet array (SDA) for dispensing one droplet into one tube. *Biosens Bioelectron*. 2023, 240: 115639. https://doi.org/10.1016/j.bios.2023.115639

RESEARCH ARTICLE

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Robust Spontaneous Raman Flow Cytometry for Single-Cell Metabolic Phenome Profiling via pDEP-DLD-RFC

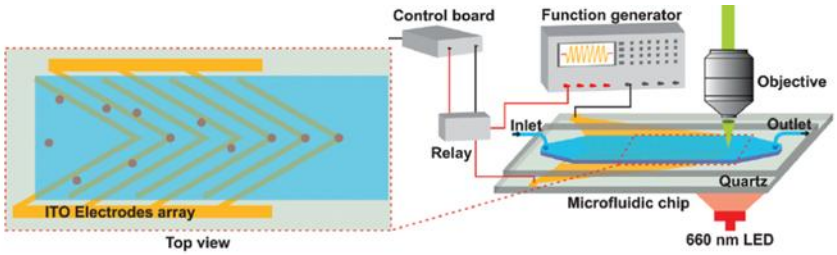
Xixian Wang, Lihui Ren, Zhidian Diao, Yuehui He, Jiaping Zhang, Min Liu, Yuandong Li, Lijun Sun, Rongze Chen, Yuetong Ji, Jian Xu, and Bo Ma**

Abstract

A full-spectrum spontaneous single-cell Raman spectrum (fs-SCRS) captures the metabolic phenome for a given cellular state of the cell in a label-free, landscape-like manner.

Herein a positive dielectrophoresis induced deterministic lateral displacement-based Raman flow cytometry (pDEP-DLD-RFC) is established. This robust flow cytometry platform utilizes a periodical positive dielectrophoresis induced deterministic lateral displacement (pDEP-DLD) force that is exerted to focus and trap fast-moving single cells in a wide channel, which enables efficient fs-SCRS acquisition and extended stable running time. It automatically produces deeply sampled, heterogeneity-resolved, and highly reproducible ramanomes for isogenic cell populations of yeast, microalgae, bacteria, and human cancers, which support biosynthetic process dissection, antimicrobial susceptibility profiling, and cell-type classification. Moreover, when coupled with intra-ramanome correlation analysis, it reveals state- and cell-type-specific metabolic heterogeneity and metabolite-conversion networks.

The throughput of ≈30–2,700 events min^{-1} for profiling both nonresonance and resonance marker bands in a fs-SCRS, plus the >5 h stable running time, represent the highest performance among reported spontaneous Raman flow cytometry (RFC) systems. Therefore, pDEP-DLD-RFC is a valuable new tool for label-free, noninvasive, and high-throughput profiling of single-cell metabolic phenomes.



Highlight

- FlowRACS is characterized by broad-spectrum applicability, high throughput, and operational stability. It can be used for tumor cell classification, microalgae biosynthesis process monitoring, multi-phenotypic monitoring of oleaginous yeast, and antimicrobial susceptibility testing (AST).

- Wang X, *et al*. Robust Spontaneous Raman Flow Cytometry for Single-Cell Metabolic Phenome Profiling via pDEP-DLD-RFC. *Adv Sci*. 2023, 10(16): e2207497.
https://doi.org/10.1002/advs.202207497

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-29337-x OPEN

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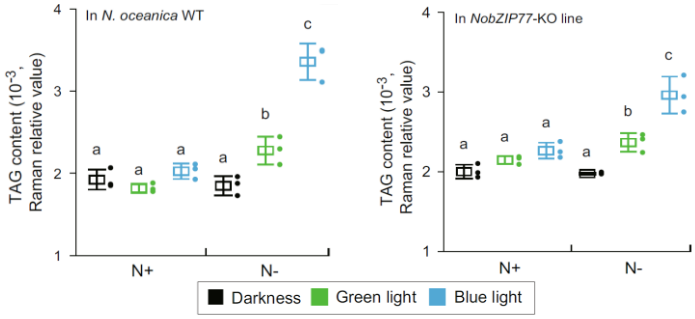
Exploring a blue-light-sensing transcription factor to double the peak productivity of oil in *Nannochloropsis oceanica*

Peng Zhang[1,2,3,6], Yi Xin[1,2,3,6], Yuehui He[1,2,3], Xianfeng Tang[3,4], Chen Shen[1,2,3], Qintao Wang[1,2,3], Nana Lv[1,2,3], Yun Li[1,2,3], Qiang Hu[2,5] & Jian Xu[1,2,3]✉

Abstract

Oleaginous microalgae can produce triacylglycerol (TAG) under stress, yet the underlying mechanism remains largely unknown.

Here, we show that, in *Nannochloropsis oceanica*, a bZIP-family regulator NobZIP77 represses the transcription of a type-2 diacylgycerol acyltransferase encoding gene *NoDGAT2B* under nitrogen-repletion (N+), while nitrogendepletion (N−) relieves such inhibition and activates *NoDGAT2B* expression and synthesis of TAG preferably from C16:1. Intriguingly, NobZIP77 is a sensor of blue light (BL), which reduces binding of NobZIP77 to the *NoDGAT2B*-promoter, unleashes NoDGAT2B and elevates TAG under N−. Under N+ and white light, *NobZIP77* knockout fully preserves cell growth rate and nearly triples TAG productivity. Moreover, exposing the *NobZIP77*-knockout line to BL under N− can double the peak productivity of TAG. These results underscore the potential of coupling light quality to oil synthesis in feedstock or bioprocess development.



Highlight

- RACS-Seq enables quantitative detection of oil (triacylglycerol, TAG) content in oleaginous microalgae *Nannochloropsis oceanica.*

- Zhang P, *et al*. Exploring a blue-light-sensing transcription factor to double the peak productivity of oil in *Nannochloropsis oceanica*. *Nat Commun*. 2022, 13(1):1664. https://doi.org/10.1038/s41467-022-29337-x

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RESEARCH ARTICLE

Intra-Ramanome Correlation Analysis Unveils Metabolite Conversion Network from an Isogenic Population of Cells

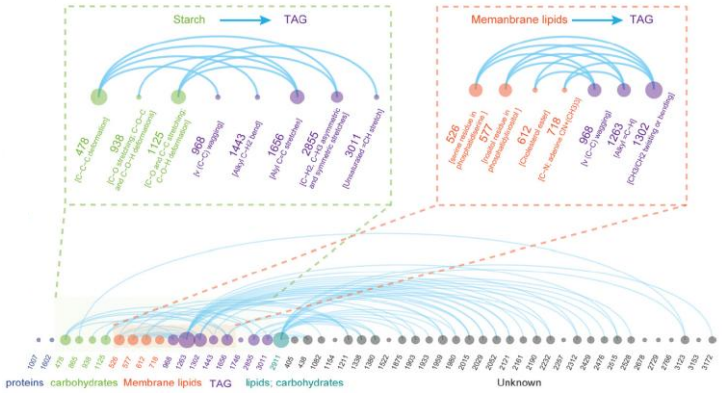
Yuehui He,[a,b,c] Shi Huang,[a,b,c,d] Peng Zhang,[a,b,c] Yuetong Ji,[a,b,c] Jian Xu[a,b,c]

Abstract

To reveal the dynamic features of cellular systems, such as the correlation among phenotypes, a time or condition series set of samples is typically required.

Here, we propose intra-ramanome correlation analysis (IRCA) to achieve this goal from just one snapshot of an isogenic population, via pairwise correlation among the cells of the thousands of Raman peaks in single-cell Raman spectra (SCRS), i.e., by taking advantage of the intrinsic metabolic heterogeneity among individual cells. For example, IRCA of *Chlamydomonas reinhardtii* under nitrogen depletion revealed metabolite conversions at each time point plus their temporal dynamics, such as protein-to-starch conversion followed by starch-to-triacylglycerol (TAG) conversion, and conversion of membrane lipids to TAG. Such among-cell correlations in SCRS vanished when the starch-biosynthesis pathway was knocked out yet were fully restored by genetic complementation.

Extension of IRCA to 64 microalgal, fungal, and bacterial ramanomes suggests the IRCA-derived metabolite conversion network as an intrinsic metabolic signature of isogenic cellular population that is reliable, species-resolved, and state-sensitive. The high-throughput, low cost, excellent scalability, and general extendibility of IRCA suggest its broad applications.



Highlight

- FlowRACS can be used to perform intra-ramanome correlation analysis (IRCA), revealing the dynamic network of metabolite transformation in *Chlamydomonas reinhardtii* under nitrogen-starved conditions.

- He Y, *et al*. Intra-Ramanome Correlation Analysis Unveils Metabolite Conversion Network from an Isogenic Population of Cells. *mBio*. 2021, 12(4): e0147021. https://doi.org/10.1128/mbio.01470-21

analytical chemistry

pubs.acs.org/ac Article

Culture-Free Identification and Metabolic Profiling of Microalgal Single Cells via Ensemble Learning of Ramanomes

Mohammadhadi Heidari Baladehi, Maryam Hekmatara, Yuehui He, Yogendra Bhaskar, Zengbin Wang, Lu Liu, Yuetong Ji, and Jian Xu*

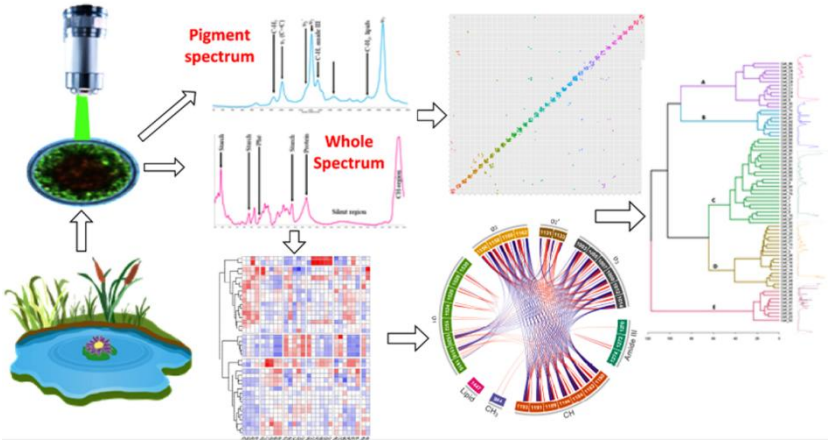
Abstract

Microalgae are among the most genetically and metabolically diverse organisms on earth, yet their identification and metabolic profiling have generally been slow and tedious.

Here, we established a reference ramanome database consisting of single-cell Raman spectra (SCRS) from >9000 cells of 27 phylogenetically diverse microalgal species, each under stationary and exponential states. When combined, prequenching ("pigment spectrum" (PS)) and postquenching ("whole spectrum" (WS)) signals can classify species and states with 97% accuracy via ensemble machine learning.

Moreover, the biosynthetic profile of Raman-sensitive metabolites was unveiled at single cells, and their interconversion was detected via intra-ramanome correlation analysis.

Furthermore, not-yet-cultured cells from the environment were functionally characterized via PS and WS and then phylogenetically identified by Raman-activated sorting and sequencing. This PS-WS combined approach for rapidly identifying and metabolically profiling single cells, either cultured or uncultured, greatly accelerates the mining of microalgae and their products.



Highlight

- RACS-Seq enables rapid identification of microalgae species, along with characterization of their metabolic functions at single-cell resolution.

- Heidari Baladehi M, *et al*. Culture-Free Identification and Metabolic Profiling of Microalgal Single Cells via Ensemble Learning of Ramanomes. *Anal Chem*. 2021, 93(25): 8872-8880. http://doi.org/10.1021/acs.analchem.1c01015

the plant journal SEB
The Plant Journal (2021) doi: 10.1111/tpj.15227

Genome engineering of *Nannochloropsis* with hundred-kilobase fragment deletions by Cas9 cleavages

Qintao Wang[1,2,3], Yanhai Gong[1,2,3], Yuehui He[1,2,3], Yi Xin[1,2,3], Nana Lv[1,2,3], Xuefeng Du[1,2,3], Yun Li[1,2,3], Byeong-ryool Jeong[1,4] and Jian Xu[1,2,3,*]

Abstract

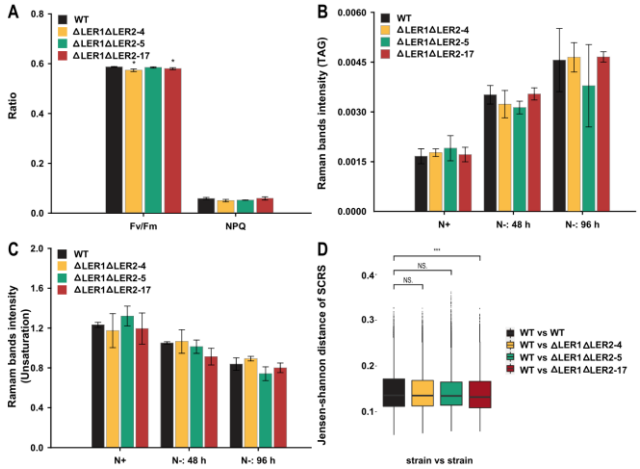
Industrial microalgae are promising photosynthetic cell factories, yet tools for large-scale targeted genome engineering are limited.

Here for the model industrial oleaginous microalga *Nannochloropsis oceanica*, we established a method to precisely and serially delete large genome fragments of ~100 kb from its 30.01 Mb nuclear genome. We started by identifying the 'non-essential' chromosomal regions (i.e. low expression region or LER) based on minimal gene expression under N-replete and N-depleted conditions.

The largest such LER (LER1) is ~98 kb in size, located near the telomere of the 502.09-kb-long Chromosome 30 (Chr 30). We deleted 81 kb and further distal and proximal deletions of up to 110 kb (21.9% of Chr 30) in LER1 by dual targeting the boundaries with the episome-based CRISPR/Cas9 system. The telomere-deletion mutants showed normal telomeres consisting of CCCTAA repeats, revealing telomere regeneration capability after losing the distal part of Chr 30. Interestingly, the deletions caused no significant alteration in growth, lipid production or photosynthesis (transcript-abundance change for < 3% genes under N depletion).

We also achieved double-deletion of both LER1 and LER2 (from Chr 9) that total ~214 kb at maximum, which can result in slightly higher growth rate and biomass productivity than the wild-type.

Therefore, loss of the large, yet 'non-essential' regions does not necessarily sacrifice important traits. Such serial targeted deletions of large genomic regions had not been previously reported in microalgae, and will accelerate crafting minimal genomes as chassis for photosynthetic production.



Highlight

- RACS-Seq can be used to detect and compare the metabolic phenotypic characteristics of wild-type and genome-edited mutants under different culture conditions.

- Wang Q, *et al*. Genome engineering of *Nannochloropsis* with hundred-kilobase fragment deletions by Cas9 cleavages. *Plant J*. 2021, 106(4): 1148-1162. https://doi.org/10.1111/tpj.15227

He *et al. Biotechnol Biofuels (2017) 10:275*
DOI 10.1186/s13068-017-0967-x

Biotechnology for Biofuels

METHODOLOGY Open Access

Label-free, simultaneous quantification of starch, protein and triacylglycerol in single microalgal cells

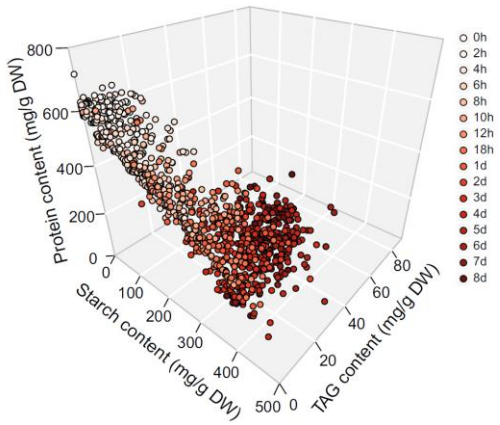
Yuehui He[1,2†], Peng Zhang[1,2†], Shi Huang[1,2], Tingting Wang[1,2], Yuetong Ji[1,2] and Jian Xu[1,2,3*]

Abstract

Background: Current approaches for quantification of major energy-storage forms in microalgae, including starch, protein and lipids, generally require cell cultivation to collect biomass followed by tedious and time-consuming analytical procedures. Thus, label-free, non-destructive and simultaneous quantification of such macromolecules at single-cell resolution is highly desirable in microalgal feedstock development and bioprocess control.

Results: Here, we established a method based on single-cell Raman spectra (SCRS) that simultaneously quantifies the contents of starch, protein, triacylglycerol (TAG) and lipid unsaturation degree in individual *Chlamydomonas reinhardtii* cells. Measurement accuracy for the contents based on full SCRS spectrum each reached 96.86–99.24%, all significantly higher than single peak-based models. However, accuracy and reliability of measurement are dependent on the number of cells sampled, thus a formal mathematical framework was proposed and validated to rationally define "minimal sampling depth" for a given state of cellular population. Furthermore, a barcode consisting of 13 marker Raman peaks was proposed to characterize the temporal dynamics of these energy-storage products, which revealed that the average contents of starch and TAG increased, while their heterogeneity indices decreased, with those of protein being exactly the opposite. Finally, our method is widely applicable, as measurements among cells from liquid suspension culture, wet paste and frozen dried powder all exhibited excellent consistency.

Conclusions: When sampled at proper depth, SCRS can serve as a quantitative and generally applicable tool for characterization and screening of strains and bioprocesses based on the profile of energy-storage macromolecules and their among-cell heterogeneity.



Highlight

- Starch, protein, and triacylglycerol (TAG) contents of individual microalgae *Chlamydomonas reinhardtii* cells during nitrogen depletion can be simultaneously quantified by single-cell Raman spectroscopy (SCRS).

- He Y, *et al*. Label-free, simultaneous quantification of starch, protein and triacylglycerol in single microalgal cells. *Biotechnol Biofuels*. 2017, 10: 275.
https://doi.org/10.1186/s13068-017-0967-x

analytical chemistry
Article
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Reverse and Multiple Stable Isotope Probing to Study Bacterial Metabolism and Interactions at the Single Cell Level

Yun Wang,[†] Yizhi Song,[‡] Yifan Tao,[§] Howbeer Muhamadali,[∥] Royston Goodacre,[∥] Ning-Yi Zhou,[⊥] Gail M. Preston,[#] Jian Xu,[*,†] and Wei E. Huang[*,‡]

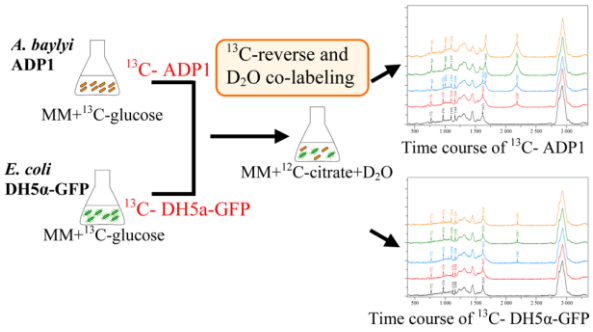
Abstract

The interactions between microorganisms driven by substrate metabolism and energy flow are important to shape diversity, abundance, and structure of a microbial community. Single cell technologies are useful tools for dissecting the functions of individual members and their interactions in microbial communities.

Here, we developed a novel Raman stable isotope probing (Raman-SIP), which uses Raman microspectroscopy coupled with reverse and D_2O colabeling to study metabolic interactions in a two-species community consisting of *Acinetobacter baylyi* ADP1 and *Escherichia coli* DH5α-GFP. This Raman-SIP approach is able to detect carbon assimilation and general metabolic activity simultaneously. Taking advantage of Raman shift of single cell Raman spectra (SCRS) mediated by incorporation of stable-isotopic substrates, Raman-SIP with reverse labeling has been applied to detect initially ^{13}C-labeled bands of ADP1 SCRS reverting back to ^{12}C positions in the presence of ^{12}C citrate. Raman-SIP with D_2O labeling has been employed to probe metabolic activity of single cells without the need of cell replication. Our results show that *E. coli* alone in minimal medium with citrate as the sole carbon source had no metabolic activity, but became metabolically active in the presence of ADP1. Mass spectrometry-based metabolite footprint analysis suggests that putrescine and phenylalanine excreted by ADP1 cells may support the metabolic activity of *E. coli*.

This study demonstrates that Raman-SIP with reverse labeling would be a useful tool to probe metabolism of any carbon substrate, overcoming limitations when stable isotopic substrates are not readily available. It is also found that Raman-SIP with D_2O labeling is a sensitive and reliable approach to distinguish metabolically active cells but not quiescent cells.

This novel approach extends the application of Raman-SIP and demonstrates its potential application as a valuable strategic approach for probing cellular metabolism, metabolic activity, and interactions in microbial communities at the single cell level.



Highlight

- The Raman stable isotope probing (Raman-SIP) technique, using ^{13}C reverse labeling and D_2O co-labeling, reveals that *E. coli* can maintain metabolic activity by utilizing metabolites produced by ADP1 during co-culture.

- Wang Y, *et al*. Reverse and Multiple Stable Isotope Probing to Study Bacterial Metabolism and Interactions at the Single Cell Level. *Anal Chem*. 2016, 88(19): 9443-9450.
https://doi.org/10.1021/acs.analchem.6b01602

Wang *et al. Biotechnology for Biofuels* 2014, **7**:58
http://www.biotechnologyforbiofuels.com/content/7/1/58

Biotechnology for Biofuels

RESEARCH Open Access

Quantitative dynamics of triacylglycerol accumulation in microalgae populations at single-cell resolution revealed by Raman microspectroscopy

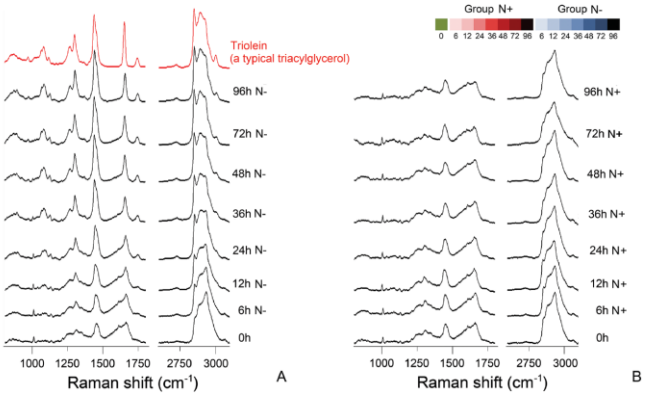
Tingting Wang[1†], Yuetong Ji[1†], Yun Wang[1], Jing Jia[1], Jing Li[1], Shi Huang[1], Danxiang Han[3], Qiang Hu[3], Wei E Huang[2*] and Jian Xu[1*]

Abstract

Background: Rapid, real-time and label-free measurement of the cellular contents of biofuel molecules such as triacylglycerol (TAG) in populations at single-cell resolution are important for bioprocess control and understanding of the population heterogeneity. Raman microspectroscopy can directly detect the changes of metabolite profile in a cell and thus can potentially serve these purposes.

Results: Single-cell Raman spectra (SCRS) of the unicellular oleaginous microalgae *Nannochloropsis oceanica* from the cultures under nitrogen depletion (TAG-producing condition) and nitrogen repletion (non-TAG-producing condition) were sampled at eight time points during the first 96 hours upon the onset of nitrogen depletion. Single *N. oceanica* cells were captured by a 532-nm laser and the SCRS were acquired by the same laser within one second per cell. Using chemometric methods, the SCRS were able to discriminate cells between nitrogen-replete and nitrogen-depleted conditions at as early as 6 hours with >93.3% accuracy, and among the eight time points under nitrogen depletion with >90.4% accuracy. Quantitative prediction of TAG content in single cells was achieved and validated via SCRS and liquid chromatography-mass spectrometry (LC-MS) analysis at population level. SCRS revealed the dynamics of heterogeneity in TAG production among cells in each isogenic population. A significant negative correlation between TAG content and lipid unsaturation degree in individual microalgae cells was observed.

Conclusions: Our results show that SCRS can serve as a label-free and non-invasive proxy for quantitatively tracking and screening cellular TAG content in real-time at single-cell level. Phenotypic comparison of single cells via SCRS should also help investigating the mechanisms of functional heterogeneity within a cellular population.



Highlight

- Microalgal oil production in each cell can be quantitatively tracked using single-cell Raman spectroscopy (SCRS).

- Wang T, *et al*. Quantitative dynamics of triacylglycerol accumulation in microalgae populations at single-cell resolution revealed by Raman microspectroscopy. *Biotechnol Biofuels*. 2014, 7:58.
https://doi.org/10.1186/1754-6834-7-58

Biotechnology Journal
www.biotechnology-journal.com
Biotechnol. J. 2014, 9
DOI 10.1002/biot.201400165

Technical Report

Raman spectroscopy provides a rapid, non-invasive method for quantitation of starch in live, unicellular microalgae

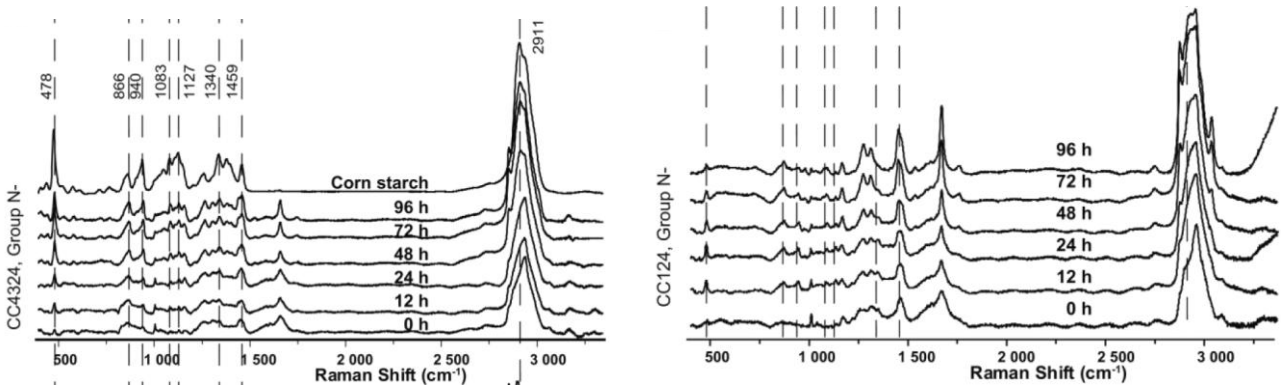
Yuetong Ji[1,], Yuehui He[1,2,*], Yanbin Cui[3], Tingting Wang[1], Yun Wang[1], Yuanguang Li[3], Wei E. Huang[4], and Jian Xu[1]*

Abstract

Conventional methods for quantitation of starch content in cells generally involve starch extraction steps and are usually labor intensive, thus a rapid and non-invasive method will be valuable. Using the starch-producing unicellular microalga *Chlamydomonas reinhardtii* as a model, we employed a customized Raman spectrometer to capture the Raman spectra of individual single cells under distinct culture conditions and along various growth stages.

The results revealed a nearly linear correlation (R^2 = 0.9893) between the signal intensity at 478 cm^{-1} and the starch content of the cells. We validated the specific correlation by showing that the starch-associated Raman peaks were eliminated in a mutant strain where the AGPase (ADP-glucose pyrophosphorylase) gene was disrupted and consequentially the biosynthesis of starch blocked.

Furthermore, the method was validated in an industrial algal strain of *Chlorella pyrenoidosa*. This is the first demonstration of starch quantitation in individual live cells. Compared to existing cellular starch quantitation methods, this single-cell Raman spectra-based approach is rapid, label-free, non-invasive, culture-independent, low-cost, and potentially able to simultaneously track multiple metabolites in individual live cells, therefore should enable many new applications.



Highlight

- Single-cell Raman spectroscopy (SCRS) enables rapid and non-invasive quantitation of starch in live microalgae at single-cell resolution.

- Ji Y, *et al*. Raman spectroscopy provides a rapid, non-invasive method for quantitation of starch in live, unicellular microalgae. *Biotechnol J*. 2014, 9(12): 1512-8. https://doi.org/10.1002/biot.201400165.

Ren *et al. Microb Cell Fact (2017) 16:233*
https://doi.org/10.1186/s12934-017-0849-8

Microbial Cell Factories

RESEARCH Open Access

Using Raman spectroscopy and chemometrics to identify the growth phase of *Lactobacillus casei* Zhang during batch culture at the single-cell level

Yan Ren[1,2], Yuetong Ji[3], Lin Teng[3] and Heping Zhang[1,2*]

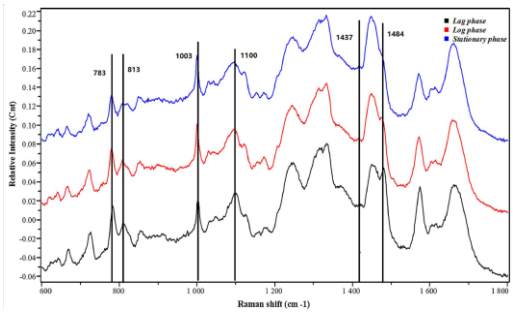
Abstract

Background: As microbial cultures are comprised of heterogeneous cells that differ according to their size and intracellular concentrations of DNA, proteins, and other constituents, the detailed identification and discrimination of the growth phases of bacterial populations in batch culture is challenging. Cell analysis is indispensable for quality control and cell enrichment.

Methods: In this paper, we report the results of our investigation on the use of single-cell Raman spectrometry (SCRS) for real-time analysis and prediction of cells in different growth phases during batch culture of *Lactobacillus (L.) casei* Zhang. A targeted analysis of defined cell growth phases at the level of the single cell, including lag phase, log phase, and stationary phase, was facilitated by SCRS.

Results: Spectral shifts were identified in different states of cell growth that reflect biochemical changes specific to each cell growth phase. Raman peaks associated with DNA and RNA displayed a decrease in intensity over time, whereas protein-specific and lipid-specific Raman vibrations increased at different rates. Furthermore, a supervised classification model (Random Forest) was used to specify the lag phase, log phase, and stationary phase of cells based on SCRS, and a mean sensitivity of 90.7% and mean specificity of 90.8% were achieved. In addition, the correct cell type was predicted at an accuracy of approximately 91.2%.

Conclusions: To conclude, Raman spectroscopy allows label-free, continuous monitoring of cell growth, which may facilitate more accurate estimates of the growth states of lactic acid bacterial populations during fermented batch culture in industry.



Highlight

- Analysis of the variations in Raman spectral peaks across three growth phases enables real-time monitoring and differentiation of growth phases for *Lactobacillus casei* Zhang during batch culture at the single-cell level.

- Ren Y, *et al*. Using Raman spectroscopy and chemometrics to identify the growth phase of *Lactobacillus casei* Zhang during batch culture at the single-cell level. *Microb Cell Fact*. 2017, 16(1): 233. https://doi.org/10.1186/s12934-017-0849-8

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Mining robust *in situ* phosphorus-accumulating organisms via single-cell RACS-Culture for rational ecosystem engineering

Xiaoyan Jing [a,b], Yanhai Gong [b,d,e,f], Yishang Ren [b,d,e,f], Liyan Wang [c], Runzhi Mu [c], Pengcheng Sun [b,d,e,f], Zhidian Diao [b,d,e,f], Yu Meng [b,d,e,f], Liming Huang [c], Xixian Wang [b,d,e,f], Jia Zhang [b,d,e,f], Jiaxuan Luan [c], Yuetong Ji [g], Bo Ma [b,d,e,f], Huihui Pan [b,d,e,f,*], Yushu Jing [c,*], Jian Xu [b,d,e,f,*]

Abstract

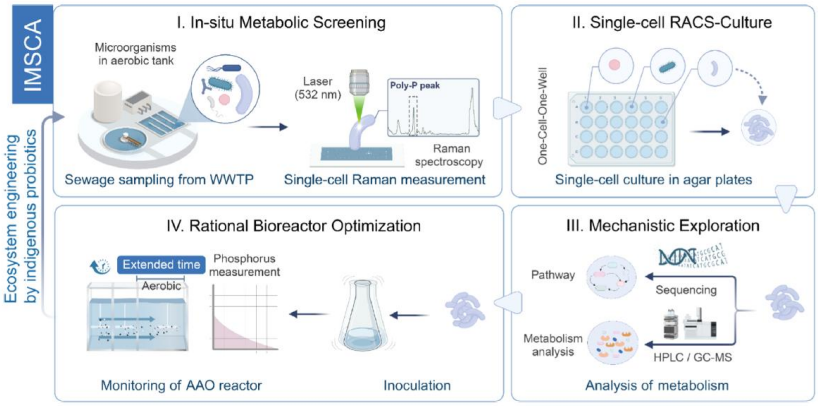
Rational engineering of ecosystems is often hindered by the inability to rapidly identify, profile, culture and apply the microbes that underlie target metabolic activity *in situ*.

Here, we developed an *In-situ* Metabolism driven Sorting, Culture and Augmentation (IMSCA) strategy via Raman-activated Cell Sorting coupled to single-cell culture (scRACS-Culture), and demonstrated it through the mining of *in situ* polyphosphate-accumulating organisms (PAOs) for wastewater treatment.

Single-cell polyphosphate-accumulating activities *in situ* were quantitatively assessed directly from environmental samples via the polyphosphate band in Raman spectrum, revealing their remarkable distinction from those from pure cultures. Among cells with the highest *in situ* activities and then sorted for one-cell-one-well cultivation are *Micrococcus luteum* CI5–8, which however shows very low activity as pure culture. This organism represents a new type of PAO due to its lack of anaerobic phosphate release, reliance on glycogen instead of polyhydroxyalkanoate as energy storage form, and incapability of denitrification.

Process redesign based on these novel physiological traits showed that time- and location-specific introduction of MCI5–8 into actual wastewater elevated phosphorus (P) removal efficiency from 45 % to 89 % in an anaerobic-anoxic-aerobic (AAO) reactor.

Therefore, by label-free profiling, sorting and cultivation of individual cells based on *in situ* metabolism in a "screen-first culture-second" manner, IMSCA is a powerful and broadly applicable strategy for efficient bioresource mining and rational ecosystem engineering.



Highlights

- RACS-Seq enables the identification, mining, and cultivation of functional microbes based on their *in situ* metabolism.
- The identified polyphosphate-accumulating organism (PAO) exhibits novel physiological traits, supporting rational ecosystem engineering through bioaugmentation.

- Jing X, *et al*. Mining robust *in situ* phosphorus-accumulating organisms via single-cell RACS-Culture for rational ecosystem engineering. *Water Res*. 2025, 284:124025. https://doi.org/10.1016/j.watres.2025.124025

ARTICLE

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Phylogeny-metabolism dual-directed single-cell genomics for dissecting and mining ecosystem function by FISH-scRACS-seq

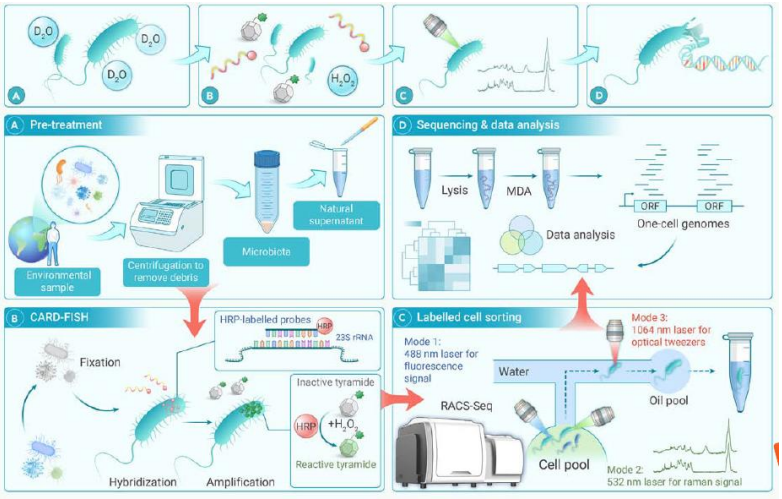
Xiaoyan Jing,[1,6,7,8,10] Yanhai Gong,[1,6,7,8,10] Zhidian Diao,[1,6,7,8,10] Yan Ma,[1,2,10] Yu Meng,[1,6,7,8] Jie Chen,[1,6,7,8] Yishang Ren,[1,6,7,8] Yuting Liang,[5] Yinchao Li,[3] Weihan Sun,[4] Jia Zhang,[1,6,7,8] Yuetong Ji,[9] Zhiqi Cong,[1,6,7,8] Shengying Li,[4] Bo Ma,[1,6,7,8] Zhisong Cui,[3,*] Li Ma,[4,*] and Jian Xu[1,6,7,8,*]

Abstract

Microbiome-wide association studies (MWASs) have uncovered microbial markers linked to ecosystem traits, but the mechanisms underlying their functions can remain elusive. This is largely due to challenges in validating their *in situ* metabolic activities and tracing such activities to individual genomes.

Here, we introduced a phylogeny-metabolism dual-directed single-cell genomics approach called fluorescence-*in situ*-hybridization-guided single-cell Raman-activated sorting and sequencing (FISH-scRACS-seq). It directly localizes individual cells from target taxon via a FISH probe for marker organism, profiles their *in situ* metabolic functions via single-cell Raman spectra, sorts cells of target taxonomy and target metabolism, and produces indexed, high-coverage, and precisely-one-cell genomes. From cyclohexane-contaminated seawater, cells representing the MWAS-derived marker taxon of γ-Proteobacteria and that are actively degrading cyclohexane *in situ* were directly identified via FISH and Raman, respectively, then sorted and sequenced for one-cell full genomes. In such a *Pseudoalteromonas fuliginea* cell, we discovered a three-component cytochrome P450 system that can convert cyclohexane to cyclohexanol *in vitro*, representing a previously unknown group of cyclohexane-degrading enzymes and organisms.

Therefore, by unveiling enzymes, pathways, genomes, and their *in situ* cellular functions specifically for those organisms with ecological relevance at one-cell resolution, FISH-scRACS-seq is a rational and generally applicable approach to dissecting and mining microbiota functions.



Highlight

- FISH-scRACS-Seq enables taxonomy-guided, *in situ* function-driven profiling of microbiota at single-cell resolution, and provides a broadly applicable approach for dissecting and mining microbiota functions.

- Jing X, *et al*. Phylogeny-metabolism dual-directed single-cell genomics for dissecting and mining ecosystem function by FISH-scRACS-seq. *The Innovation*. 2025, 6(3): 100759. https://doi.org/10.1016/j.xinn.2024.100759.

analytical chemistry

pubs.acs.org/ac Article

Artificial Intelligence-Assisted Automatic Raman-Activated Cell Sorting (AI-RACS) System for Mining Specific Functional Microorganisms in the Microbiome

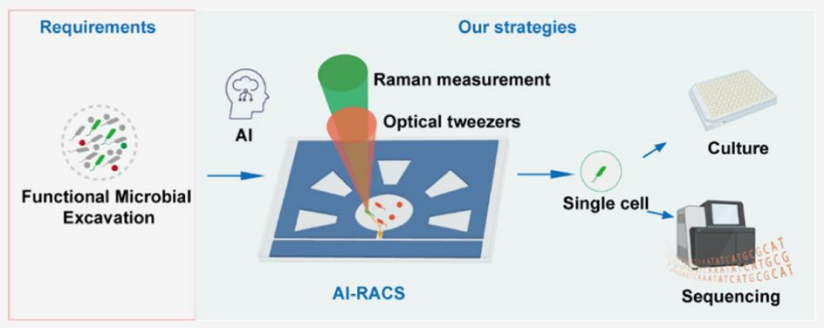
Zhidian Diao,[▽] Xiaoyan Jing,[▽] Xibao Hou,[▽] Yu Meng, Jiaping Zhang, Yongshun Wang, Yuetong Ji, Anle Ge, Xixian Wang, Yuting Liang,* Jian Xu,* and Bo Ma*

Abstract

The microbiome represents the natural presence of microorganisms, and exploring, understanding, and leveraging its functions will bring about significant breakthroughs in life sciences and applications.

Raman-activated cell sorting (RACS) enables the correlation of phenotype and genotype at the single-cell level, offering a solution to the bottleneck in microbial community functional analysis caused by challenges in cultivating diverse microorganisms. However, current labor-intensive manual procedures fall short in catering to the demands of single-cell functional analysis in microbial communities. To address this issue, we developed an artificial intelligence-assisted Raman-activated cell sorting system (AI-RACS) that integrates precise single-cell positioning, automated data collection, optical tweezers capture, and single-cell printing to elevate microbial single-cell RACS from manual to automated, validating the efficacy of the system by isolating aluminum-tolerant microbes from acidic soil microbiota.

Leveraging the AI-RACS framework, we sorted 13 strains from red soil samples under near-in situ conditions, with all demonstrating strong aluminum tolerance. AI-RACS efficiently segregates microbial cells from intricate environmental samples, investigating their functional attributes and presenting a novel tool for microbial research and applications.



Highlight

- RACS-Seq integrates single-cell positioning, automated functional data acquisition, optical tweezers-based cell capture, and single-cell printing into a fully automated workflow, as demonstrated by the isolation of aluminum-tolerant microbes from acidic soil microbiota.

- Diao Z, *et al*. Artificial Intelligence-Assisted Automatic Raman-Activated Cell Sorting (AI-RACS) System for Mining Specific Functional Microorganisms in the Microbiome. *Analytical Chemistry*. 2024, 96(46): 18416-18426. https://pubs.acs.org/doi/10.1021/acs.analchem.4c03213

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Magnetic nanoparticle-mediated enrichment technology combined with microfluidic single cell separation technology: A technology for efficient separation and degradation of functional bacteria in single cell liquid phase

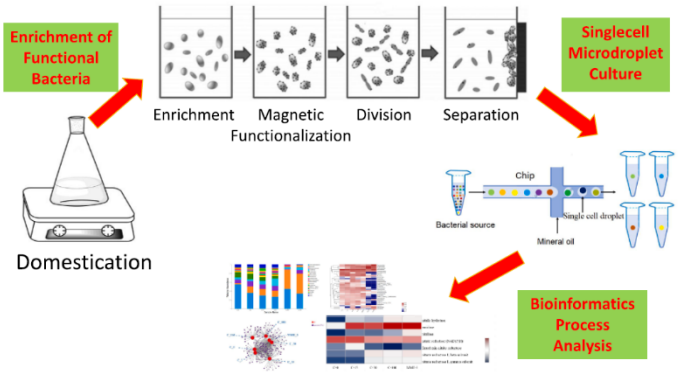
Yuanyan Xuan [a], Meng Yin [a], Yujiao Sun [a,*], Meijun Liu [a], Guomin Bai [a], Zhidian Diao [b,c,*], Bo Ma [b,c]

Abstract

Although there are many microorganisms in nature, the limitations of isolation and cultivation conditions have restricted the development of artificial enhanced remediation technology using functional microbial communities.

In this study, an integrated technology of Magnetic Nanoparticle-mediated Enrichment (MME) and Microfluidic Single Cell separation (MSC) that breaks through the bottleneck of traditional separation and cultivation techniques and can efficiently obtain more in situ functional microorganisms from the environment was developed. MME technology was first used to enrich rapidly growing active bacteria in the environment. Subsequently, MSC technology was applied to isolate and incubate functional bacterial communities in situ and validate the degradation ability of individual bacteria.

As a result, this study has changed the order of traditional pure culture methods, which are first selected and then cultured, and provided a new method for obtaining non-culturable functional microorganisms.



Highlight

- EasySort enables direct isolation of live cells from magnetic nanoparticle-treated samples without the need for cultivation. The isolated cells are subsequently expanded in culture, and their total DNA is extracted and sequenced for identification.

- Xuan Y, *et al*. Magnetic nanoparticle-mediated enrichment technology combined with microfluidic single cell separation technology: A technology for efficient separation and degradation of functional bacteria in single cell liquid phase. *Bioresour Technol*. 2024, 401: 130686. https://doi.org/10.1016/j.biortech.2024.130686

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Cycloalkane degradation by an uncultivated novel genus of Gammaproteobacteria derived from China's marginal seas

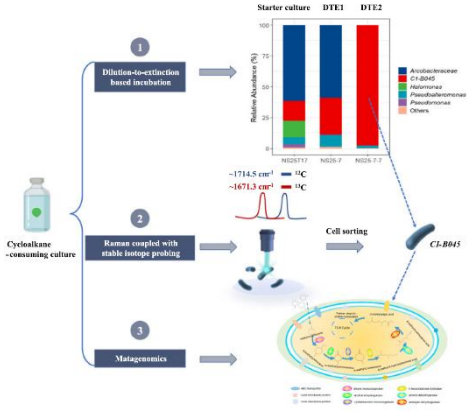
Zhisong Cui [a,*], Yingchao Li [a], Xiaoyan Jing [b], Xiao Luan [c], Na Liu [d], Jinyan Liu [a], Yu Meng [b], Jian Xu [b], David L. Valentine [d,*]

Abstract

The consumption of cycloalkanes is prevalent in low-temperature marine environments, likely influenced by psychrophilic microorganisms. Despite their significance, the primary active species responsible for marine cycloalkane degradation remain largely unidentified due to cultivation challenges.

In this study, we provide compelling evidence indicating that the uncultured genus C1-B045 of Gammaproteobacteria is a pivotal participant in cycloalkane decomposition within China's marginal seas. Notably, the relative abundance of C1-B045 surged from 15.9% in the methylcyclohexane (MCH)-consuming starter culture to as high as 97.5% in MCH-utilizing extinction cultures following successive dilution-to-extinction and incubation cycles. We used stable isotope probing, Raman-activated gravity-driven encapsulation, and 16 S rRNA gene sequencing to link cycloalkane-metabolizing phenotype to genotype at the single-cell level.

By annotating key enzymes (e.g., alkane monooxygenase, cyclohexanone monooxygenase, and 6-hexanolactone hydrolase) involved in MCH metabolism within C1-B045's representative metagenome-assembled genome, we developed a putative MCH degradation pathway.



Highlight

- RACS-Seq reveals a distinct red shift in single-cell Raman spectra of C1-B045 bacteria following ^{13}C-Cyclohexane (^{13}C-CH) substrate feeding, demonstrating their active metabolic engagement in MCH degradation.

- Cui Z, *et al*. Cycloalkane degradation by an uncultivated novel genus of Gammaproteobacteria derived from China's marginal seas. *Journal of Hazardous Materials*. 2024, 469: 133904. https://doi.org/10.1016/j.jhazmat.2024.133904

AAAS
BioDesign Research
Volume 2022, Article ID 9782712, 16 pages
https://doi.org/10.34133/2022/9782712

BioDesign Research
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Research Article

Revealing CO_2-Fixing SAR11 Bacteria in the Ocean by Raman-Based Single-Cell Metabolic Profiling and Genomics

Xiaoyan Jing,[1,2,3] Yanhai Gong,[1,3] Teng Xu,[1,3] Paul A. Davison,[4] Craig MacGregor-Chatwin,[4] C. Neil Hunter,[4] La Xu,[5] Yu Meng,[1,3] Yuetong Ji,[1,3,6] Bo Ma,[1,3] Jian Xu,[1,2,3] and Wei E. Huang[7]

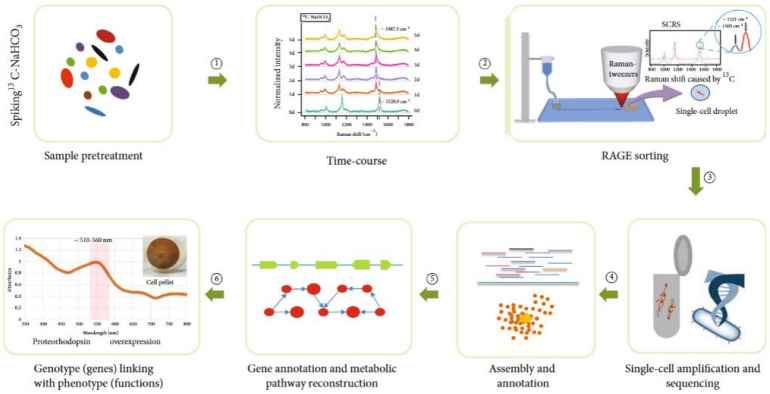
Abstract

The majority of marine microbes remain uncultured, which hinders the identification and mining of CO_2-fixing genes, pathways, and chassis from the oceans.

Here, we investigated CO_2-fixing microbes in seawater from the euphotic zone of the Yellow Sea of China by detecting and tracking their ^{13}C-bicarbonate (^{13}C-HCO_3^-) intake via single-cell Raman spectra (SCRS) analysis. The target cells were then isolated by Raman-activated Gravity-driven Encapsulation (RAGE), and their genomes were amplified and sequenced at one-cell resolution.

The single-cell metabolism, phenotype and genome are consistent. We identified a not-yet-cultured *Pelagibacter* spp., which actively assimilates ^{13}C-HCO_3^-, and also possesses most of the genes encoding enzymes of the Calvin-Benson cycle for CO_2 fixation, a complete gene set for a rhodopsin-based light-harvesting system, and the full genes necessary for carotenoid synthesis.

The four proteorhodopsin (PR) genes identified in the *Pelagibacter* spp. were confirmed by heterologous expression in *E. coli*. These results suggest that hitherto uncultured *Pelagibacter* spp. uses light-powered metabolism to contribute to global carbon cycling.



Highlight

- RACS-Seq identified target cells based on the ^{13}C-induced Raman peak shifts in their single-cell Raman spectra (SCRS), and subsequently sorted them in a ”one cell per tube” format.

- Jing X, *et al*. Revealing CO_2-Fixing SAR11 Bacteria in the Ocean by Raman-Based Single-Cell Metabolic Profiling and Genomics. *Biodes Res*. 2022, 2022: 9782712. https://doi.org/10.34133/2022/9782712

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ARTICLE OPEN

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Single-cell Raman-activated sorting and cultivation (scRACS-Culture) for assessing and mining in situ phosphate-solubilizing microbes from nature

Xiaoyan Jing[1,2,3,4,9], Yanhai Gong[1,2,3,4,9], Huihui Pan[1,2,3,4], Yu Meng[1,2,3,4], Yishang Ren[1,2,3,4], Zhidian Diao[1,2,3,4], Runzhi Mu[5], Teng Xu[1,2,3,4], Jia Zhang[1,2,3,4], Yuetong Ji[1,3,4,6], Yuandong Li[1,2,3,4], Chen Wang[1,2,3,4], Lingyun Qu[7], Li Cui[8], Bo Ma[1,2,3,4]✉ and Jian Xu[1,2,3,4]✉

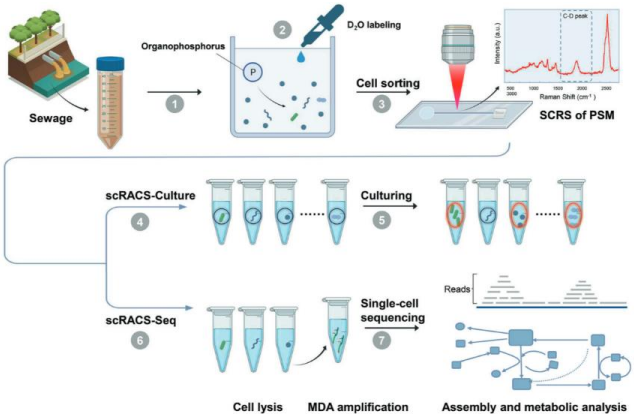
Abstract

Due to the challenges in detecting in situ activity and cultivating the not-yet-cultured, functional assessment and mining of living microbes from nature has typically followed a 'culture-first' paradigm. Here, employing phosphate-solubilizing microbes (PSM) as model, we introduce a 'screen-first' strategy that is underpinned by a precisely one-cell-resolution, complete workflow of single-cell Raman-activated Sorting and Cultivation (scRACS-Culture).

Directly from domestic sewage, individual cells were screened for in-situ organic-phosphate-solubilizing activity via D_2O intake rate, sorted by the function via Raman-activated Gravity-driven Encapsulation (RAGE), and then cultivated from precisely one cell. By scRACS-Culture, pure cultures of strong organic PSM including *Comamonas* spp., *Acinetobacter* spp., *Enterobacter* spp. and *Citrobacter* spp., were derived, whose phosphate-solubilizing activities in situ are 90-200% higher than in pure culture, underscoring the importance of 'screen-first' strategy.

Moreover, employing scRACS-Seq for post-RACS cells that remain uncultured, we discovered a previously unknown, low-abundance, strong organic-PSM of *Cutibacterium* spp. that employs secretary metallophosphoesterase (MPP), cell-wall-anchored 5'-nucleotidase (encoded by *ushA*) and periplasmic-membrane located PstSCAB-PhoU transporter system for efficient solubilization and scavenging of extracellular phosphate in sewage.

Therefore, scRACS-Culture and scRACS-Seq provide an *in situ* function-based, “screen-first” approach for assessing and mining microbes directly from the environment.



Highlight

- RACS-Seq enables the discovery and mining of organic phosphate-solubilizing microbes in sewage by establishing genotype-metabolic activity linkages at single-cell resolution through activity-guided sorting and culture-based verification.

- Jing X, *et al*. Single-cell Raman-activated sorting and cultivation (scRACS-Culture) for assessing and mining in situ phosphate-solubilizing microbes from nature. *ISME Commun*. 2022, 2(1): 106. https://doi.org/10.1038/s43705-022-00188-3

ORIGINAL RESEARCH

Engineering archaeal membrane-spanning lipid GDGT biosynthesis in bacteria: Implications for early life membrane transformations

Huahui Chen[1], Fengfeng Zheng[1], Xi Feng[1], Zijing Huang[1], Wei Yang[1], Chuanlun Zhang[1], Wenbin Du[2], Kira S. Makarova[3], Eugene V. Koonin[3], and Zhirui Zeng[1,*]

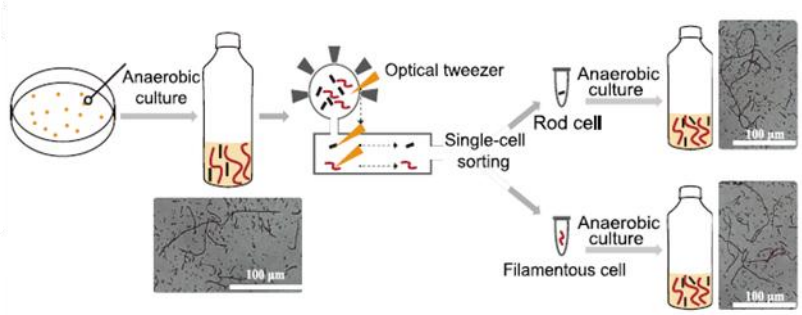
mLife

Abstract

Eukaryotes are hypothesized to be archaeal–bacterial chimeras. Given the different chemical structures of membrane phospholipids in archaea and bacteria, transformations of membranes during eukaryogenesis that led to the bacterial-type membranes of eukaryotic cells remain a major conundrum. One of the possible intermediates of eukaryogenesis could involve an archaeal–bacterial hybrid membrane. So far, organisms with hybrid membranes have not been discovered, and experimentation on such membranes has been limited.

To generate mixed membranes, we reconstructed the archaeal membrane lipid biosynthesis pathway in *Escherichia coli*, creating three strains that individually produced archaeal lipids ranging from simple, such as DGGGOH (digeranylgeranylglycerol) and archaeol, to complex, such as GDGT (glycerol dialkyl glycerol tetraether). The physiological responses became more pronounced as the hybrid membrane incorporated more complex archaeal membrane lipids. In particular, biosynthesis of GDGT induced a pronounced SOS response, accompanied by cellular filamentation, explosive cell lysis, and ATP accumulation.

Thus, bacteria seem to be able to incorporate simple archaeal membrane lipids, such as DGGGOH and archaeol, without major fitness costs, compatible with the involvement of hybrid membranes at the early stages of cell evolution and in eukaryogenesis. By contrast, the acquisition of more complex, structurally diverse membrane lipids, such as GDGT, appears to be strongly deleterious to bacteria, suggesting that this type of lipid is an archaeal innovation.



Highlight

- EasySort enables precise morphological sorting of functional single cells using optical tweezers.

- Chen H, *et al*. Engineering archaeal membrane-spanning lipid GDGT biosynthesis in bacteria: implications for early life membrane transformations. *mLife*. 2025, 4(2): 193-204. https://doi.org/10.1002/mlf2.70001.

Artificial intelligence-assisted automatic and index-based microbial single-cell sorting system for One-Cell-One-Tube

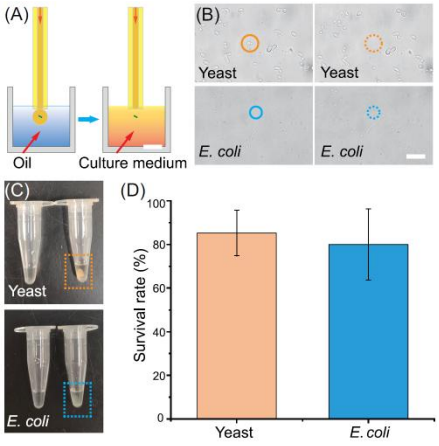
Zhidian Diao[1,2,3,4,#], Lingyan Kan[1,3,4,#], Yilong Zhao[1,3,4,#], Huaibo Yang[5], Jingyun Song[1,3,4], Chen Wang[1,3,4], Yang Liu[1,3,4], Fengli Zhang[1,3,4], Teng Xu[1,2,3,4], Rongze Chen[1,2,3,4], Yuetong Ji[1,5], Xixian Wang[1,2,3,4], Xiaoyan Jing[1,2,3,4], Jian Xu[1,2,3,4], Yuandong Li[1,2,3,4,*], and Bo Ma[1,2,3,4,*]

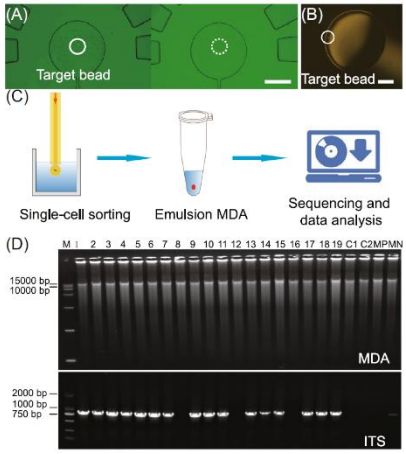
Abstract

Identification, sorting, and sequencing of individual cells directly from in situ samples have great potential for in-depth analysis of the structure and function of microbiomes.

In this work, based on an artificial intelligence (AI)-assisted object detection model for cell phenotype screening and a cross-interface contact method for single-cell exporting, we developed an automatic and index-based system called EasySort AUTO, where individual microbial cells are sorted and then packaged in a microdroplet and automatically exported in a precisely indexed, "One-Cell-One-Tube" manner. The target cell is automatically identified based on an AI-assisted object detection model and then mobilized via an optical tweezer for sorting. Then, a cross-interface contact microfluidic printing method that we developed enables the automated transfer of cells from the chip to the tube, which leads to coupling with subsequent single-cell culture or sequencing. The efficiency of the system for single-cell printing is >93%. The throughput of the system for single-cell printing is ~120 cells/h. Moreover, >80% of single cells of both yeast and *Escherichia coli* are culturable, suggesting the superior preservation of cell viability during sorting.

Finally, AI-assisted object detection supports automated sorting of target cells with high accuracy from mixed yeast samples, which was validated by downstream single-cell proliferation assays. The automation, index maintenance, and vitality preservation of EasySort AUTO suggest its excellent application potential for single-cell sorting.





Highlight

- EasySort enables the isolation and sorting of single cells for downstream applications such as single-cell genome analysis and cultivation.

- Diao Z, *et al*. Artificial intelligence-assisted automatic and index-based microbial single-cell sorting system for One-Cell-One-Tube. *mLife*. 2022, 1(4): 448-459.
https://doi.org/10.1002/mlf2.12047

The ISME Journal
https://doi.org/10.1038/s41396-021-00950-w

ISME

ARTICLE

Microbial metabolism and necromass mediated fertilization effect on soil organic carbon after long-term community incubation in different climates

Haowei Ni[1,2] · Xiaoyan Jing[3] · Xian Xiao[1] · Na Zhang[1,2] · Xiaoyue Wang[1] · Yueyu Sui[4] · Bo Sun[1] · Yuting Liang[1,2]

Abstract

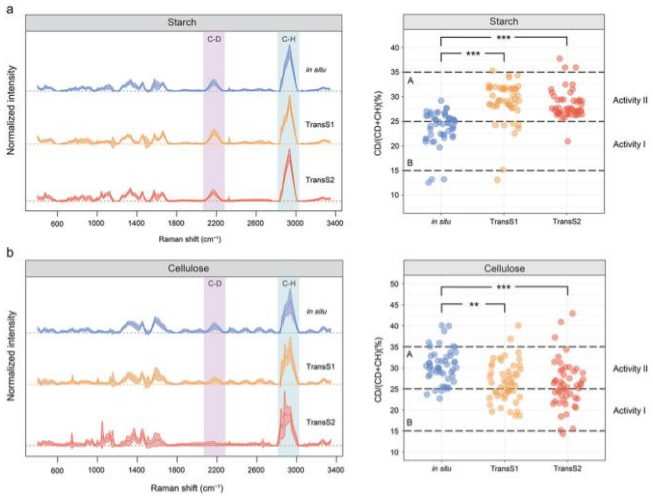
Understanding the effects of changing climate and long-term human activities on soil organic carbon (SOC) and the mediating roles of microorganisms is critical to maintain soil C stability in agricultural ecosystem.

Here, we took samples from a long-term soil transplantation experiment, in which large transects of Mollisol soil in a cold temperate region were translocated to warm temperate and mid-subtropical regions to simulate different climate conditions, with a fertilization treatment on top.

This study aimed to understand fertilization effect on SOC and the role of soil microorganisms featured after long-term community incubation in warm climates. After 12 years of soil transplantation, fertilization led to less reduction of SOC, in which aromatic C increased and the consumption of O-alkyl C and carbonyl C decreased. Soil live microbes were analyzed using propidium monoazide to remove DNAs from dead cells, and their network modulization explained 60.4% of variations in soil labile C.

Single-cell Raman spectroscopy combined with D_2O isotope labeling indicated a higher metabolic activity of live microbes to use easily degradable C after soil transplantation. Compared with non-fertilization, there was a significant decrease in soil α- and β-glucosidase and delay on microbial growth with fertilization in warmer climate.

Moreover, fertilization significantly increased microbial necromass as indicated by amino sugar content, and its contribution to soil resistant C reached 22.3%. This study evidentially highlights the substantial contribution of soil microbial metabolism and necromass to refractory C of SOC with addition of nutrients in the long-term.



Highlight

- D_2O isotope labeling combined with single-cell Raman spectroscopy was used to study the metabolic activities of soil bacteria utilizing different carbon sources (starch and cellulose) in fertilized soil.

- Ni H, *et al*. Microbial metabolism and necromass mediated fertilization effect on soil organic carbon after long-term community incubation in different climates. *ISME J*. 2021, 15(9): 2561-2573. https://doi.org/10.1038/s41396-021-00950-w

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RESEARCH ARTICLE

One-Cell Metabolic Phenotyping and Sequencing of Soil Microbiome by Raman-Activated Gravity-Driven Encapsulation (RAGE)

Xiaoyan Jing,[a,c,e] Yanhai Gong,[a,c,e] Teng Xu,[a,c,e] Yu Meng,[a,c,e] Xiao Han,[a,c,e] Xiaolu Su,[a,c,e] Jianmei Wang,[a,c,e] Yuetong Ji,[a,d,e] Yuandong Li,[a,c,e] Zhongjun Jia,[b,e] Bo Ma,[a,c,e] Jian Xu[a,c,e]

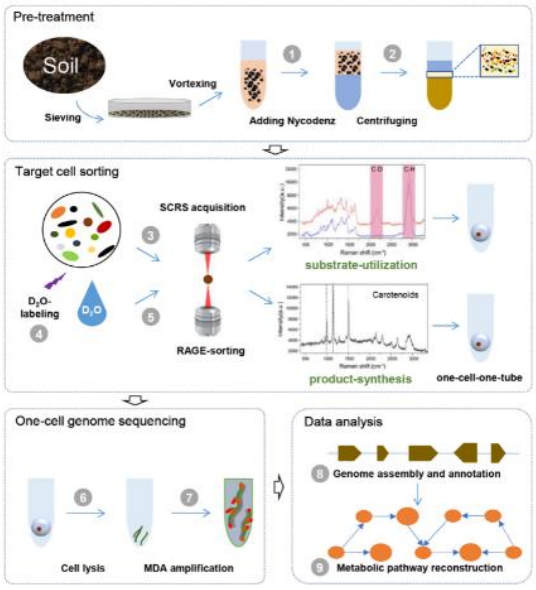
Abstract

Soil harbors arguably the most metabolically and genetically heterogeneous microbiomes on Earth, yet establishing the link between metabolic functions and genome at the precisely one-cell level has been difficult.

Here, for mock microbial communities and then for soil microbiota, we established a Raman-activated gravity-driven single-cell encapsulation and sequencing (RAGE-Seq) platform, which identifies, sorts, and sequences precisely one bacterial cell via its anabolic (incorporating D from heavy water) and physiological (carotenoid-containing) functions.

We showed that (i) metabolically active cells from numerically rare soil taxa, such as *Corynebacterium* spp., *Clostridium* spp., *Moraxella* spp., *Pantoea* spp., and *Pseudomonas* spp., can be readily identified and sorted based on D_2O uptake, and their one-cell genome coverage can reach ~93% to allow high-quality genome-wide metabolic reconstruction; (ii) similarly, carotenoid-containing cells such as *Pantoea* spp., *Legionella* spp., *Massilia* spp., *Pseudomonas* spp., and *Pedobacter* spp. were identified and one-cell genomes were generated for tracing the carotenoid-synthetic pathways; and (iii) carotenoid-producing cells can be either metabolically active or inert, suggesting culture-based approaches can miss many such cells.

As a Raman-activated cell sorter (RACS) family member that can establish a metabolism-genome link at exactly one-cell resolution from soil, RAGE-Seq can help to precisely pinpoint "who is doing what" in complex ecosystems.



Highlight

- RACS-Seq links soil microbial genotypes to phenotypes through Raman-activated, gravity-assisted cell sorting and sequencing.

- Jing X, *et al*. One-Cell Metabolic Phenotyping and Sequencing of Soil Microbiome by Raman-Activated Gravity-Driven Encapsulation (RAGE). *mSystems*. 2021, 6(3): e0018121. https://doi.org/10.1128/msystems.00181-21

Helicobacter

ORIGINAL ARTICLE

Rapid Antimicrobial Susceptibility Test of *Helicobacter pylori* to Metronidazole via Single-Cell Raman Spectrometry

Lu Sun[1] | Min Liu[2] | Yanan Gong[1] | Kangle Zhai[1,3] | FengYun Lv[2,4] | Lihua He[1] | Xinguang Xue[2] | Xiaolu Liu[2] | Hairui Wang[1] | Dongjie Fan[1] | Yuanhai You[1] | Mengyang Fang[1,5] | Luyang Sun[2,6] | Jian Xu[2,6] | Jianzhong Zhang[1]

Abstract

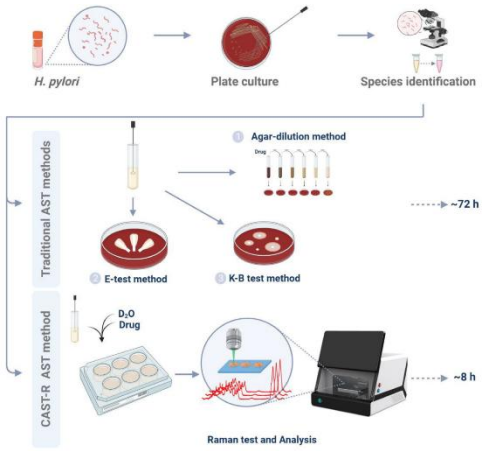
Background: Metronidazole is a first-line antibiotic to treat *Helicobacter pylori* infections. However, the Clinical Laboratory Standards Institute guidelines recommend against using antimicrobial susceptibility test (AST) to test metronidazole resistance, due to the unreliable predictive power which can result in treatment failure.

Objectives: The aim of this study was to establish an 8-h, metabolic-phenotype based AST for *H. pylori* metronidazole susceptibility using D_2O-probed Raman microspectroscopy.

Methods: Minimal inhibitory concentration (MIC) measured by conventional AST (E-test) were compared with expedited MIC via metabolic activity (eMIC-MA) for 10 *H. pylori* isolates. Raman barcodes of cellular-response to stress (RBCS) incorporating protein and carbohydrate Raman bands, were utilized to identify a biomarker to distinguish metronidazole susceptibility.

Results: Specifically, eMIC-MA produces metronidazole susceptibility results showing 100% agreement with E-test, and determines the bactericidal dosage for both high- and low-level resistant *H. pylori* strains. In addition, RBCS not just reliably distinguish between metronidazole-susceptible and -resistant strains, but reveal their distinct mechanisms in bacterial responses to metronidazole.

Conclusion: The speed, accuracy, low cost, and rich information content that reveals the mode-of-action of drugs suggest the method's value in guiding metronidazole prescriptions for *H. pylori* eradication and in rapid screening based on drug-resistance mechanism.



Highlight

- RACS-Seq enables rapid metabolite-based profiling to assess metronidazole susceptibility in *H. pylori*, demonstrating full concordance with conventional methods. Furthermore, Raman barcodes of cellular-response to stress (RBCS) analysis not only distinguishes resistant and sensitive strains but also reveals heterogeneous molecular mechanisms underlying resistance.

- Sun L, *et al*. Rapid Antimicrobial Susceptibility Test of *Helicobacter pylori* to Metronidazole via Single-Cell Raman Spectrometry. *Helicobacter*. 2024, 29(5): e13136.
https://doi.org/10.1111/hel.13136

Ren *et al. Annals of Clinical Microbiology and Antimicrobials* (2023) 22:94
https://doi.org/10.1186/s12941-023-00644-5

Annals of Clinical Microbiology and Antimicrobials

RESEARCH Open Access

Rapid *Mycobacterium abscessus* antimicrobial susceptibility testing based on antibiotic treatment response mapping via Raman Microspectroscopy

Weicong Ren[1†], Yuli Mao[2,3†], Shanshan Li[1†], Bo Gao[2,3], Xiaoting Fu[2,3], Xiaolu Liu[2,3], Pengfei Zhu[2,4], Yuanyuan Shang[1], Yuandong Li[2,3], Bo Ma[2,3], Luyang Sun[2,3*], Jian Xu[2,3*] and Yu Pang[1*]

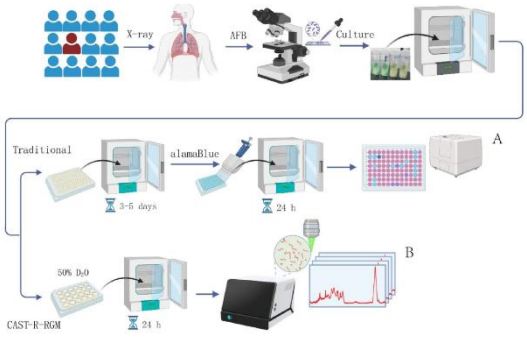
Abstract

Objectives Antimicrobial susceptibility tests (ASTs) are pivotal tools for detecting and combating infections caused by multidrug-resistant rapidly growing mycobacteria (RGM) but are time-consuming and labor-intensive.

Design We used a *Mycobacterium abscessus*-based RGM model to develop a rapid (24-h) AST from the beginning of the strain culture, the Clinical Antimicrobials Susceptibility Test Ramanometry for RGM (CAST-R-RGM). The ASTs obtained for 21 clarithromycin (CLA)-treated and 18 linezolid (LZD)-treated RGM isolates.

Results CAST-R-RGM employs D_2O-probed Raman microspectroscopy to monitor RGM metabolic activity, while also revealing bacterial antimicrobial drug resistance mechanisms. The results of clarithromycin (CLA)-treated and linezolid (LZD)-treated RGM isolates exhibited 90% and 83% categorical agreement, respectively, with conventional AST results of the same isolates. Furthermore, comparisons of time- and concentration-dependent Raman results between CLA-and LZD-treated RGM strains revealed distinct metabolic profiles after 48-h and 72-h drug treatments, despite similar profiles obtained for both drugs after 24-h treatments.

Conclusions Ultimately, the rapid, accurate, and low-cost CAST-R-RGM assay offers advantages over conventional culture-based ASTs that warrant its use as a tool for improving patient treatment outcomes and revealing bacterial drug resistance mechanisms.



Highlight

- RACS-Seq enables CAST-R-RGM, a rapid drug susceptibility assay for fast-growing mycobacteria such as *Mycobacterium abscessus*. By combining D_2O labeling with Raman spectroscopy, it reduces the detection time from 3–5 days to 24 hours.

- Ren W, *et al*. Rapid *Mycobacterium abscessus* antimicrobial susceptibility testing based on antibiotic treatment response mapping via Raman Microspectroscopy. *Ann Clin Microbiol Antimicrob*. 2023, 22(1): 94.
https://doi.org/10.1186/s12941-023-00644-5

METHOD

Rapid, automated, and reliable antimicrobial susceptibility test from positive blood culture by CAST-R

Pengfei Zhu[1,2,#], Lihui Ren[1,2,3,#], Ying Zhu[4,5,#], Jing Dai[1,2,#], Huijie Liu[1,2], Yuli Mao[1,2], Yuandong Li[1,2], Yuehui He[1,2], Xiaoshan Zheng[1,2], Rongze Chen[1,2], Xiaoting Fu[1,2], Lili Zhang[1,2], Lijun Sun[1,2], Yuanqi Zhu[6], Yuetong Ji[1,7], Bo Ma[1,2], Yingchun Xu[4], Jian Xu[1,2,8,*], and Qiwen Yang[4,*]

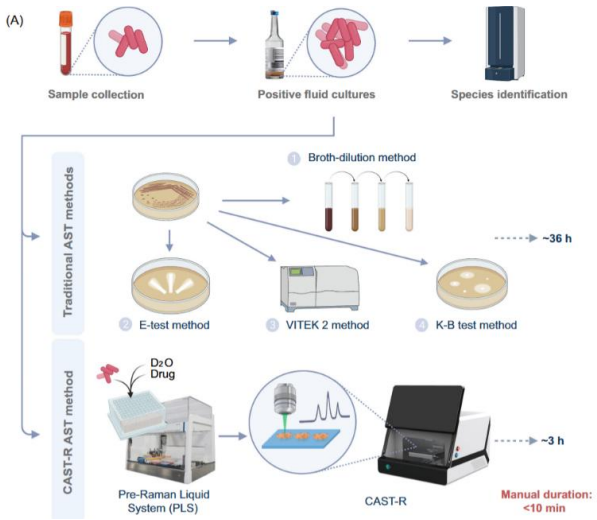
Abstract

Antimicrobial susceptibility tests (ASTs) are pivotal in combating multidrug resistant pathogens, yet they can be time-consuming, labor-intensive, and unstable. Using the AST of tigecycline for sepsis as the main model, here we establish an automated system of Clinical Antimicrobials Susceptibility Test Ramanometry (CAST-R), based on D_2O-probed Raman microspectroscopy.

Featuring a liquid robot for sample pretreatment and a machine learning-based control scheme for data acquisition and quality control, the 3-h, automated CAST-R process accelerates AST by >10-fold, processes 96 paralleled antibiotic-exposure reactions, and produces high-quality Raman spectra.

The Expedited Minimal Inhibitory Concentration via Metabolic Activity is proposed as a quantitative and broadly applicable parameter for metabolism-based AST, which shows 99% essential agreement and 93% categorical agreement with the broth microdilution method (BMD) when tested on 100 *Acinetobacter baumannii* isolates.

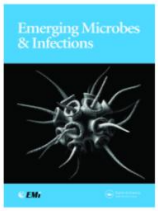
Further tests on 26 clinically positive blood samples for eight antimicrobials, including tigecycline, meropenem, ceftazidime, ampicillin/sulbactam, oxacillin, clindamycin, vancomycin, and levofloxacin reveal 93% categorical agreement with BMD-based results. The automation, speed, reliability, and general applicability of CAST-R suggest its potential utility for guiding the clinical administration of antimicrobials.



Highlight

- The rapid, automated, and reliable CAST-R workflow for antimicrobial susceptibility test (AST) showed excellent agreement with conventional AST culture-based workflows, and was validated using clinical blood samples.

- Zhu P, *et al*. Rapid, automated, and reliable antimicrobial susceptibility test from positive blood culture by CAST-R. *mLife*. 2022, 1(3): 329-340.
https://doi.org/10.1002/mlf2.12019



Emerging Microbes & Infections

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Novel tigecycline resistance mechanisms in *Acinetobacter baumannii* mediated by mutations in *adeS*, *rpoB* and *rrf*

Xiaoting Hua, Jintao He, Jingfen Wang, Linghong Zhang, Linyue Zhang, Qingye Xu, Keren Shi, Sebastian Leptihn, Yue Shi, Xiaoting Fu, Pengfei Zhu, Paul G. Higgins & Yunsong Yu

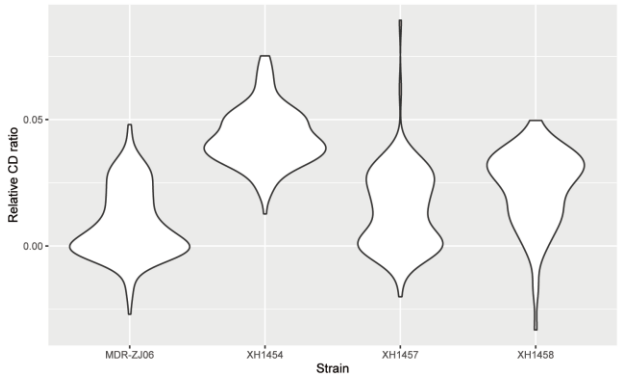
Abstract

Acinetobacter baumannii is an important pathogen in hospital acquired infections. Although tigecycline currently remains a potent antibiotic for treating infections caused by multidrug resistant *A. baumannii* (MDRAB) strains, reports of tigecycline resistant isolates have substantially increased. The resistance mechanisms to tigecycline in *A. baumannii* are far more complicated and diverse than what has been described in the literature so far.

Here, we characterize *in vitro*-selected MDRAB strains obtained by increasing concentrations of tigecycline. We have identified mutations in *adeS*, *rrf* and *rpoB* that result in reduced susceptibility to tigecycline. Using *in situ* complementation experiments, we confirm that mutations in *rrf*, *rpoB*, and two types of mutations in *adeS* correlate with tigecycline resistance.

By Western blot and polysome profile analysis, we demonstrate that the *rrf* mutation results in decreased expression of RRF, which affects the process of ribosome recycling ultimately leading to increased tigecycline tolerance. A transcriptional analysis shows that the mutated *rpoB* gene plays a role in regulating the expression of the SAM-dependent methyltransferase (*trm*) and transcriptional regulators, to confer moderate tigecycline resistance.

This study provides direct *in vitro* evidence that mutations in the *adeS*, *rpoB* and *rrf* are associated with tigecycline resistance in *A. baumannii*.



Highlight

- RACS-Seq can detect subtle differences in drug sensitivity across strains with closely related MIC values.

- Hua X, *et al*. Novel tigecycline resistance mechanisms in *Acinetobacter baumannii* mediated by mutations in *adeS*, *rpoB* and *rrf*. *Emerg Microbes Infect*. 2021, 10(1): 1404-1417. https://doi.org/10.1080/22221751.2021.1948804

analytical chemistry

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D_2O-Probed Raman Microspectroscopy Distinguishes the Metabolic Dynamics of Macromolecules in Organellar Anticancer Drug Response

Maryam Hekmatara,* Mohammadhadi Heidari Baladehi, Yuetong Ji, and Jian Xu*

Abstract

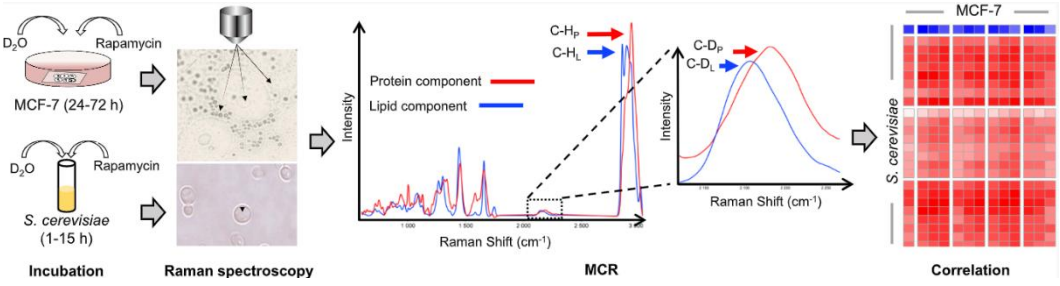
To profile the metabolic dynamics responding to drugs at the single-cell/organelle resolution, rapid and economical mechanism-revealing methods are required.

Here, we introduced D_2O-probed Raman microspectroscopy in combination with the multivariate curve resolution-alternating least squares (MCR-ALS or MCR) algorithm.

Exploiting MCR to deconvolute each macromolecular component specifically, the method is able to track and distinguish changes in lipid and protein metabolic activities in a human cancer cell line (MCF-7) and in *Saccharomyces cerevisiae*, in response to the metabolism-inhibitory effect of rapamycin, which inhibits the mammalian/mechanistic target of rapamycin (mTOR) signaling. Under rapamycin, in the lipid bodies of cancer cells, metabolic activities of both protein and lipid are suppressed; in the nucleus, protein synthesis remains active, whereas lipid synthesis is inhibited; in the cytoplasm, syntheses of protein and lipid are both dose- and duration-dependent.

Thus, rapamycin differentially influences protein and lipid synthesis in mTOR signaling. Moreover, the strong correlation between macromolecular-specific components of yeast and those in MCF-7 cytoplasm, nucleus, and lipid bodies revealed similarity in rapamycin response. Notably, highly metabolically active cancer cells after high-dosage rapamycin exposure (500 or 5000 × IC_{50}) were revealed, which escape detection by population-level cytotoxicity tests.

Thus, by unveiling macromolecule-specific metabolic dynamics at the organelle level, the method is valuable to mechanism-based rapid screening and dissection of drug response.



Highlight

- RACS-Seq enables rapid and cost-effective detection of tumor drug sensitivity at single-organelle resolution via metabolic inhibition, while simultaneously revealing underlying drug resistance mechanisms.

- Hekmatara M, *et al*. D_2O-Probed Raman Microspectroscopy Distinguishes the Metabolic Dynamics of Macromolecules in Organellar Anticancer Drug Response. *Anal Chem*. 2021, 93(4): 2125-2134. https://doi.org/10.1021/acs.analchem.0c03925

analytical chemistry
Article
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Metabolic-Activity-Based Assessment of Antimicrobial Effects by D_2O-Labeled Single-Cell Raman Microspectroscopy

Yifan Tao,[†,‡] Yun Wang,[‡,∥] Shi Huang,[‡,∥] Pengfei Zhu,[‡,∥] Wei E Huang,[§] Junqi Ling,[*,†] and Jian Xu[*,‡,∥]

Abstract

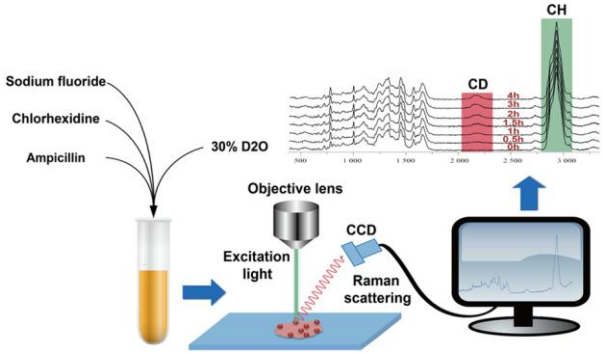
To combat the spread of antibiotic resistance, methods that quantitatively assess the metabolism-inhibiting effects of drugs in a rapid and culture-independent manner are urgently needed.

Here using four oral bacteria as models, we show that heavy water (D_2O)-based single-cell Raman microspectroscopy (D_2O-Raman) can probe bacterial response to different drugs using the Raman shift at the C−D (carbon− deuterium vibration) band in 2040 to 2300 cm^{-1} as a universal biomarker for metabolic activity at single-bacterial-cell resolution.

The "minimum inhibitory concentration based on metabolic activity" (MIC-MA), defined as the minimal dose under which the median ΔC−D-ratio at 8 h of drug exposure is ≤0 and the standard deviation (SD) of the ΔC−D ratio among individual cells is ≤0.005, was proposed to evaluate the metabolism-inhibiting efficacy of drugs.

In addition, heterogeneity index of MIC-MA (MIC-MA-HI), defined as SD of C−D ratio among individual cells, quantitatively assesses the among-cell heterogeneity of metabolic activity after drug regimens. When exposed to 1× MIC of sodium fluoride (NaF), 1× MIC of chlorhexidine (CHX), or 60× MIC of ampicillin, the cariogenic oral pathogen *Streptococcus mutans* UA159 ceased propagation yet remained metabolically highly active.

This underscores the advantage of MIC-MA over the growth-based MIC in being able to detect the "nongrowing but metabolically active" (NGMA) cells that underlie many latent or recurring infections. Moreover, antibiotic susceptible and resistant *S. mutans* strains can be readily discriminated at as early as 0.5 h. Thus, D_2O-Raman can serve as a universal method for rapid and quantitative assessment of antimicrobial effects based on general metabolic activity at single-cell resolution.



Highlight

- RACS-Seq enables rapid, culture-free detection of antibiotic susceptibility at single-cell resolution by monitoring bacterial metabolic activities through Raman signals of carbon-deuterium (C-D) bonds.

- Tao Y, *et al*. Metabolic-Activity-Based Assessment of Antimicrobial Effects by D_2O-Labeled Single-Cell Raman Microspectroscopy. *Anal Chem*. 2017, 89(7): 4108-4115.
https://doi.org/10.1021/acs.analchem.6b05051

SCIENTIFIC REPORTS

OPEN

Label-free, rapid and quantitative phenotyping of stress response in *E. coli* via ramanome

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Accepted: 13 September 2016

Lin Teng[1,2], Xian Wang[1,2], Xiaojun Wang[1,2], Honglei Gou[1], Lihui Ren[1], Tingting Wang[1], Yun Wang[1], Yuetong Ji[1], Wei E. Huang[3] & Jian Xu[1]

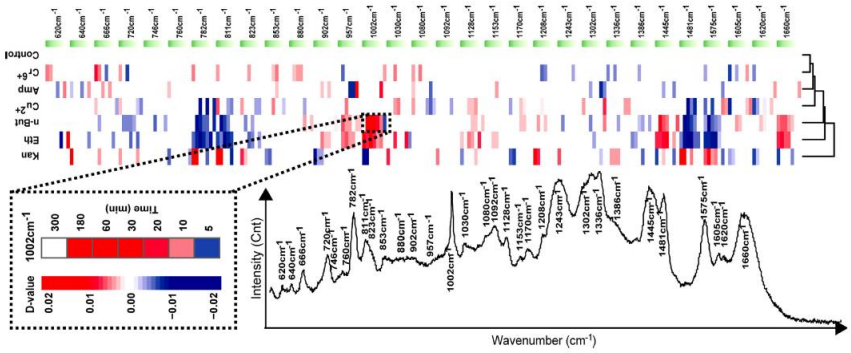
Abstract

Rapid profiling of stress-response at single-cell resolution yet in a label-free, non-disruptive and mechanism-specific manner can lead to many new applications.

We propose a single-cell-level biochemical fingerprinting approach named “ramanome”, which is the collection of Single-cell Raman Spectra (SCRS) from a number of cells randomly selected from an isogenic population at a given time and condition, to rapidly and quantitatively detect and characterize stress responses of cellular population. SCRS of *Escherichia coli* cells are sensitive to both exposure time (eight time points) and dosage (six doses) of ethanol, with detection time as early as 5 min and discrimination rate of either factor over 80%.

Moreover, the ramanomes upon six chemical compounds from three categories, including antibiotics of ampicillin and kanamycin, alcohols of ethanol and n-butanol and heavy metals of Cu^{2+} and Cr^{6+}, were analyzed and 31 marker Raman bands were revealed which distinguish stress-responses via cytotoxicity mechanism and variation of inter-cellular heterogeneity.

Furthermore, specificity, reproducibility and mechanistic basis of ramanome were validated by tracking stress-induced dynamics of metabolites and by contrasting between cells with and without genes that convey stress resistance. Thus ramanome enables rapid prediction and mechanism-based screening of cytotoxicity and stress-response programs at single-cell resolution.



Highlight

- Raman-barcode of cellular-response to stressors (RBCS) allows rapid, label-free, and quantitative profiling of cytotoxicity and stress response in cell populations at single-cell resolution.

- Teng L, et al. Label-free, rapid and quantitative phenotyping of stress response in *E. coli* via ramanome. *Sci Rep*. 2016, 6: 34359. https://doi.org/10.1038/srep34359

Clinical Chemistry 00:0
1–11 (2022)

Molecular Diagnostics and Genetics

Single-Cell Identification, Drug Susceptibility Test, and Whole-genome Sequencing of *Helicobacter pylori* Directly from Gastric Biopsy by Clinical Antimicrobial Susceptibility Test Ramanometry

Min Liu,[a,b,†] Pengfei Zhu,[a,b,†] Lei Zhang,[a,b,†] Yanhai Gong,[a,b] Chen Wang,[a,b] Lu Sun,[c] Lili Wang,[d] Rongze Chen,[a,b] Yuli Mao,[a,b] Xiaoting Fu,[a,b] Lili Zhang,[a,b] Teng Xu,[a,b] Yuetong Ji,[a,e] Quanjiang Dong,[d] Bo Ma,[a,b] Jianzhong Zhang,[c,*] and Jian Xu [a,b,f,*]

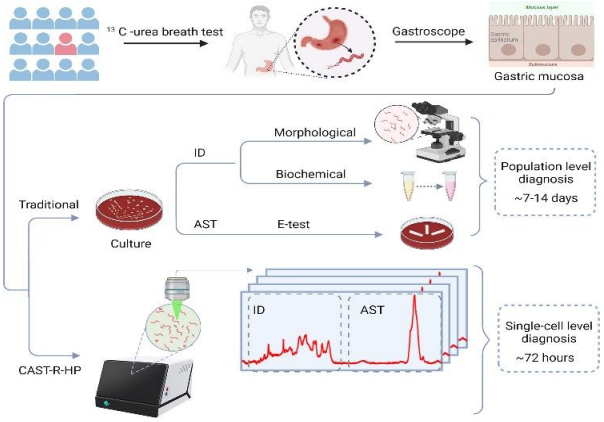
Abstract

Background: The battle against *Helicobacter pylori* (*H. pylori*) infections demands fast, reliable, and sensitive methods for pathogen identification (ID), antimicrobial susceptibility tests (ASTs) based on metabolic response, and genome-wide mutation profiling that reveals resistance mechanisms.

Methods: Here we introduce Clinical Antimicrobial Susceptibility Test Ramanometry for *H. pylori* (CAST-R-HP), and its validation with clinical samples. This method performs rapid ID, metabolism inhibition–based AST, and high-quality whole-genome sequencing for cells of targeted resistance phenotype, all at precisely 1-cell resolution and directly from biopsy samples.

Results: In CAST-R-HP, automated acquisition and machine learning of single-cell Raman spectra (SCRS) enable distinguishing individual *H. pylori* cells directly from a biopsy sample, with 98.5 ± 0.27% accuracy in ID. Moreover, by adding a 48- to72-h D_2O feeding and drug exposure step prior to SCRS acquisition, CAST-R-HP reports AST for levofloxacin and clarithromycin with 100% accuracy, based on metabolic inhibition level. Furthermore, CAST-R-HP supports rapid sorting, low-bias DNA amplification, and full genome sequencing of single *H. pylori* cells with the SCRS defined, targeted drug-susceptibility phenotype, via Raman-activated gravity-driven cell encapsulation and sequencing. The genome-wide mutation map (maximum 99.70% coverage), at precisely 1-cell resolution, not only elucidates the drug-susceptibility phenotypes but also unveils their underlying molecular mechanisms.

Conclusion: The culture independency, shorter turnaround time, high resolution, and comprehensive information output suggest that CAST-R-HP is a powerful tool for diagnosing and treating *H. pylori* infections.



Highlight

- The CAST-R-HP workflow for rapid pathogen identification and antimicrobial susceptibility test (AST) at single-cell resolution directly from clinical gastric biopsy specimens, providing a powerful tool for diagnosing and treating *H. pylori* infections.

- Liu M, *et al*. Single-Cell Identification, Drug Susceptibility Test, and Whole-genome Sequencing of *Helicobacter pylori* Directly from Gastric Biopsy by Clinical Antimicrobial Susceptibility Test Ramanometry. *Clin Chem*. 2022, 68(8):1064-1074. https://doi.org/10.1093/clinchem/hvac082

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Phenome–Genome Profiling of Single Bacterial Cell by Raman-Activated Gravity-Driven Encapsulation and Sequencing

Teng Xu, Yanhai Gong, Xiaolu Su, Pengfei Zhu, Jing Dai, Jian Xu, and Bo Ma**

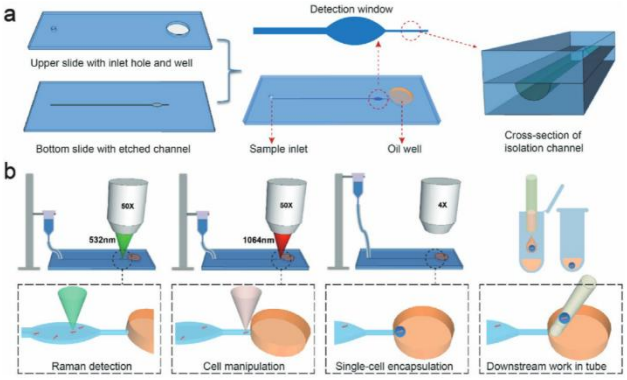
Abstract

The small size and low DNA amount of bacterial cells have hindered establishing phenome-genome links in a precisely indexed, one-cell-per-reaction manner.

Here, Raman-Activated Gravity-driven single-cell Encapsulation and Sequencing (RAGE-Seq) is presented, where individual cells are phenotypically screened via single-cell Raman spectra (SCRS) in an aquatic, vitality-preserving environment, then the cell with targeted SCRS is precisely packaged in a picoliter microdroplet and readily exported in a precisely indexed, "one-cell-one-tube" manner. Such integration of microdroplet encapsulation to Raman-activated sorting ensures high-coverage one-cell genome sequencing or cultivation that is directly linked to metabolic phenotype. For clinical *Escherichia coli* isolates, genome assemblies derived from precisely one cell via RAGE-Seq consistently reach >95% coverage.

Moreover, directly from a urine sample of urogenital tract infection, metabolic-activity-based antimicrobial susceptibility phenotypes and genome sequence of 99.5% coverage are obtained simultaneously from precisely one cell.

This single-cell global mutation map corroborates resistance phenotype and genotype, and unveils epidemiological features with high specificity and sensitivity. The ability to profile and correlate bacterial metabolic phenome and high-quality genome sequences at one-cell resolution suggests broad application of RAGE-Seq.



Highlight

- RACS-Seq enables discrimination of pathogenic bacterial species at single-cell resolution, detection of drug susceptibility, and high-coverage genomic sequencing directly from clinical urine samples.

- Xu T, *et al*. Phenome-Genome Profiling of Single Bacterial Cell by Raman-Activated Gravity-Driven Encapsulation and Sequencing. *Small*. 2020, 16(30): e2001172.
https://doi.org/10.1002/smll.202001172

frontiers | Frontiers in Microbiology

Single-cell Raman spectroscopy identifies *Escherichia coli* persisters and reveals their enhanced metabolic activities

Chuan Wang[1†], Rongze Chen[2,3†], Jian Xu[2*] and Lijian Jin[1*]

Abstract

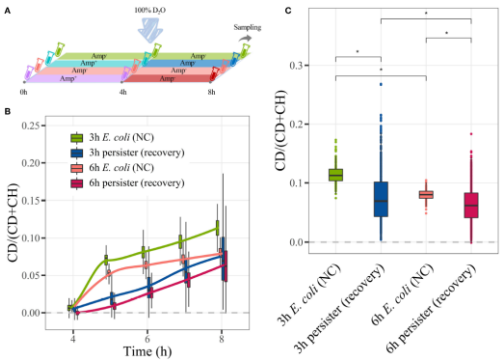
Microbial persisters are the featured tiny sub-population of microorganisms that are highly tolerant to multiple antimicrobials. Currently, studies on persisters remain a considerable challenge owing to technical limitations.

Here, we explored the application of single-cell Raman spectroscopy (SCRS) in the investigation of persisters. *Escherichia coli* (ATCC 25922) cells were treated with a lethal dosage of ampicillin (100 μg/mL, 32 × MIC, 4 h) for the formation of persisters. The biochemical characters of *E. coli* and its persisters were assessed by SCRS, and their metabolic activities were labeled and measured with D_2O-based single-cell Raman spectroscopy (D_2O-Ramanometry).

Notable differences in the intensity of Raman bands related to major cellular components and metabolites were observed between *E. coli* and its ampicillin-treated persisters. Based on their distinct Raman spectra, *E. coli* and its persister cells were classified into different projective zones through the principal component analysis and t-distributed stochastic neighbor embedding.

According to the D_2O absorption rate, *E. coli* persisters exhibited higher metabolic activities than those of untreated *E. coli*. Importantly, after the termination of ampicillin exposure, these persister cells showed a temporal pattern of D_2O intake that was distinct from non-persister cells. To our knowledge, this is the first report on identifying *E. coli* persisters and assessing their metabolic activities through the integrated SCRS and D_2O-Ramanometry approach.

These novel findings enhance our understanding of the phenotypes and functionalities of microbial persister cells. Further investigations could be extended to other pathogens by disclosing microbial pathogenicity mechanisms for developing novel therapeutic strategies and approaches.



Highlight

- RACS-Seq allows for the time-resolved measurement of cellular metabolic activity during the resuscitation of *Escherichia coli* persister cells.

- Wang C, *et al*. Single-cell Raman spectroscopy identifies *Escherichia coli* persisters and reveals their enhanced metabolic activities. *Front Microbiol*. 2022, 13: 936726. https://doi.org/10.3389/fmicb.2022.936726

Contents lists available at ScienceDirect

Sensors and Actuators: B. Chemical

journal homepage: www.elsevier.com/locate/snb

Nature-inspired fungal fusarinine C-powered dual-mode fluorometric/colorimetric biosensing of fecal *Helicobacter pylori* with high-reliability

Zhuangzhuang Wang [a,1], Mingxia Zhao [a,1], Yuan Wang [a], Bo Ma [b], Lili Wang [c], Yuetong Ji [d], Yuanyuan Ding [a,*], Zhe Chi [a,*]

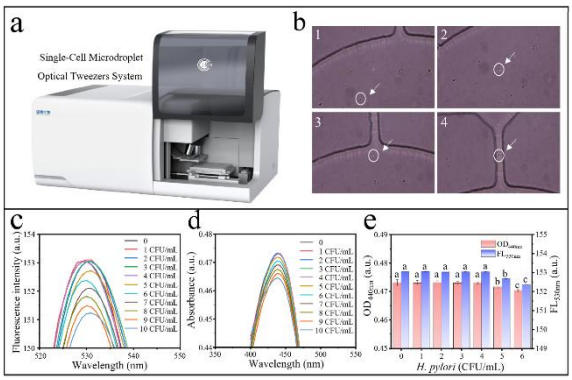
Abstract

Conventional diagnostic approaches for *Helicobacter pylori* lack sufficient sensitivity, accuracy, and flexibility, especially for low-density *H. pylori* infection. To overcome this limitation, we developed a highly reliable fluorometric/colorimetric dual-mode biosensing technique to detect minute quantities of *H. pylori* in human stool samples.

Inspired by natural porphyrins, we discovered that the water-soluble fungal siderophore fusarinine C (FsC) emitted intense green fluorescence owing to intramolecular through-bond and intermolecular through-space conjugation, and turned reddish upon coordination with Fe^{3+}. With FsC as an intrinsic fluorometric/colorimetric probe, a dual-mode biosensor was developed. It included an enrichment module of truncated *H. pylori*-specific-aptamer-modified superparamagnetic nanoparticles to capture *H. pylori* from samples, and a transduction module of alginate conjugated with FsC[Fe^{3+}] and an optimized amount of FsC without Fe^{3+} to amplify the captured *H. pylori* to fluorescent and/or color signals. Remarkably, significantly increased sensitivity was achieved for this biosensor, with an actual limit of detection of 5 CFU/mL and single-cell-level detection resolution, as validated using laser-tweezer-sorted single *H. pylori* cells.

Moreover, highly reliable dual-mode detection of low-density *H. pylori* (between 5 and 10 CFU/mL) was demonstrated. Using the colorimetric mode, a smartphone-biosensor platform for sensitive point-of-care testing of fecal *H. pylori* within 20 min was demonstrated, outperforming the clinical fecal antigen test strips; meanwhile, the fluorometric mode of the biosensor, owing to its greater sensitivity, can verify the colorimetric detection of minute *H. pylori*.

This study first showcased a FsC-powered dual-mode biosensor for ultra-sensitive and accurate point-of-care detection of *H. pylori* with high reliability.



Highlight

- EasySort enables the precise sorting of 1-10 bacterial cells to validate the actual limit of detection (LOD) of a dual-mode biosensor.

- Wang Z, *et al*. Nature-inspired fungal fusarinine C-powered dual-mode fluorometric/colorimetric biosensing of fecal *Helicobacter pylori* with high-reliability. *Sensors and Actuators B: Chemical*. 2024, 412, 135838. https://doi.org/10.1016/j.snb.2024.135838

Lab on a Chip

Versatile, facile and low-cost single-cell isolation, culture and sequencing by optical tweezer-assisted pool-screening†

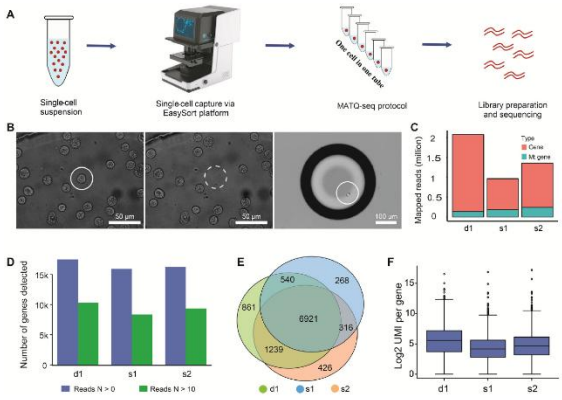
Teng Xu, ‡[abc] Yuandong Li,‡[abc] Xiao Han,[af] Lingyan Kan,[abc] Jing Ren,[abc] Luyang Sun,[abc] Zhidian Diao,[abc] Yuetong Ji,[ae] Pengfei Zhu,[ae] Jian Xu*[abcd] and Bo Ma*[abcd]

Abstract

Real-time image-based sorting of target cells in a precisely indexed manner is desirable for sequencing or cultivating individual human or microbial cells directly from clinical or environmental samples; however, the versatility of existing methods is limited as they are usually not broadly applicable to all cell sizes.

Here, an optical tweezer-assisted pool-screening and single-cell isolation (OPSI) system is established for precise, indexed isolation of individual bacterial, yeast or human-cancer cells. A controllable static flow field that acts as a cell pool is achieved in a microfluidics chip, to enable precise and ready screening of cells of 1 to 40 μm in size by bright-field, fluorescence, or Raman imaging. The target cell is then captured by a 1064 nm optical tweezer and deposited as one-cell-harboring nanoliter microdroplets in a one-cell-one-tube manner. For bacterial, yeast and human cells, OPSI achieves a >99.7% target-cell sorting purity and a 10-fold elevated speed of 10–20 cells per min.

Moreover, OPSI-based one-cell RNA-seq of human cancer cells yields high quality and reproducible single-cell transcriptome profiles. The versatility, facileness, flexibility, modularized design, and low cost of OPSI suggest its broad applications for image-based sorting of target cells.



Highlight

- EasySort enables efficient single-cell isolation for high-quality scRNA-seq analysis, as demonstrated by high gene detection rates, low mitochondrial read contamination, and consistent read coverage across samples.

- Xu T, *et al*. Versatile, facile and low-cost single-cell isolation, culture and sequencing by optical tweezer-assisted pool-screening. *Lab Chip*. 2022, 23(1): 125-135.
https://doi.org/10.1039/d2lc00888b

frontiers in Cellular and Infection Microbiology

Assessing Efficacy of Clinical Disinfectants for Pathogenic Fungi by Single-Cell Raman Microspectroscopy

Fan Li[1,2,3†], Lihui Ren[4,5,6†], Rongze Chen[4,5], Xi Sun[7,8], Jian Xu[4,5], Pengfei Zhu[4,5] and Fang Yang[1,2*]*

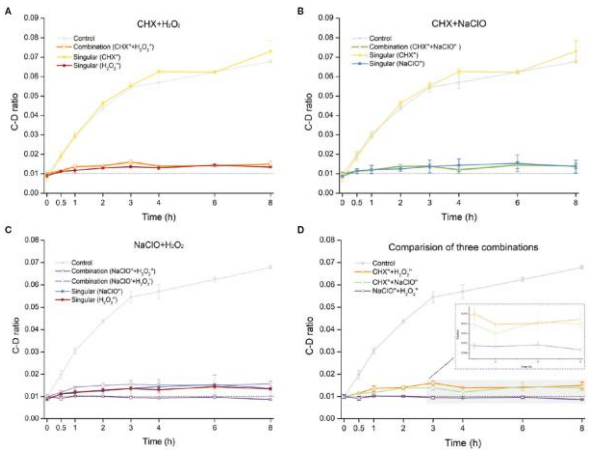
Abstract

Disinfectants are crucial for root canal therapy (RCT), as metabolism of canal-inhabiting microbes can cause refractory infections. To develop effective yet patient- and environment-friendly disinfectant formulations, we quantitatively assessed the metabolism-inhibiting effects of intracanal disinfectants via D_2O-probed Single-Cell Raman Spectra (SCRS), using *Candida albicans* (*C. albicans*) as a pathogen model.

For chlorhexidine gluconate (CHX), sodium hypochlorite (NaClO), and hydrogen peroxide (H_2O_2), at their MIC of 4, 168, and 60 μg/ml, respectively, despite the complete growth halt, metabolic activity of individual fungal cells was reduced on average by 0.4%, 93.9%, and 94.1% at 8 h, revealing a "nongrowing but metabolically active" (NGMA) state that may underlie potential refractory infections, particularly for CHX.

In contrast, at their Metabolic Activity-based Minimum Inhibitory Concentrations (MIC-MA) of 8, 336, and 120 μg/mL, respectively, metabolic activity of all cells was completely halted throughout 8 h exposure. Moreover, combined use of NaClO+H_2O_2 (combination at 0.5×MIC-MA each) outperforms solo uses of CHX, NaClO, H_2O_2, or other binary combinations.

Furthermore, dynamics of SCRS revealed distinct fungicidal mechanisms of CHX, NaClO, H_2O_2, and their pairwise combinations. MIC-MA is advantageous in critically assessing antifungal efficacy, and NaClO+H_2O_2 can potentially serve as a more efficient disinfectant formula for fungal pathogens.



Highlight

- RACS-Seq enables the comparison of metabolic activity inhibition in *Candida albicans* cells under various disinfectant combinations by simultaneously resolving the temporal dynamics of the C-D ratio under disinfectant exposure.

- Li F, *et al*. Assessing Efficacy of Clinical Disinfectants for Pathogenic Fungi by Single-Cell Raman Microspectroscopy. *Front Cell Infect Microbiol*. 2022, 12: 772378. https://doi.org/10.3389/fcimb.2022.772378

RESEARCH ARTICLE

Journal of RAMAN SPECTROSCOPY WILEY

Single-cell Raman microspectroscopy-based assessment of three intracanal disinfectants' effect on *Enterococcus faecalis*

Yuhan Liu[1,2] | Yuying Ma[2,3] | Lijuan Zhang[4] | Xi Sun[5,6] | Jiazhen Yang[2,3] | Xiuzhen Li[1,2] | Fan Li[7] | Rongze Chen[8] | Pengfei Zhu[8] | Jian Xu[8] | Fang Yang[1,2]

Abstract

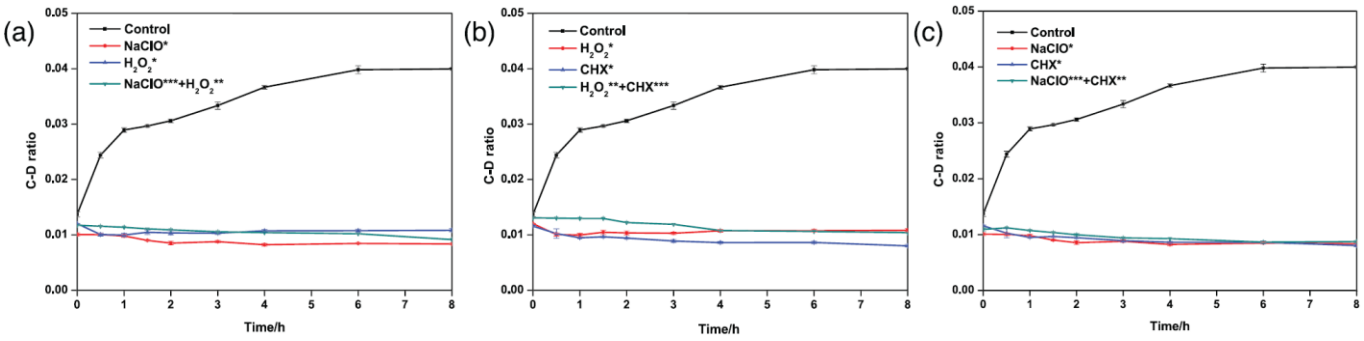
Enterococcus faecalis (*E. faecalis*) is frequently encountered in asymptomatic, persistent endodontic infections; thus, its control and eradication via disinfectants are important. To explore a disinfectant formulation that is effective yet with minimal side effects, here, we evaluated the susceptibility of *E. faecalis* to three intracanal disinfectants.

We quantitatively assessed and compared the growth- or metabolism-inhibiting effects of intracanal disinfectants via minimum inhibitory concentration (MIC) and minimum inhibitory concentration based on metabolic activity (MIC-MA), based on the broth dilution test and D_2O-probed single-cell Raman spectra (SCRS), respectively.

For sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2), and chlorhexidine gluconate (CHX), the corresponding MIC was 0.45 g/L, 110 mg/L, and 6 mg/L, respectively. Under their respective MIC doses, metabolic activity of bacterial cells was reduced to 2%, 4%, and 2% remained at 8 h, yet recovered to a retention level of 90%, 97%, and 2% at 24 h.

Despite the halting of growth, the remained metabolic activity suggests a "nongrowing but metabolically active" (NGMA) state that may lead to potential recurring infections. In contrast, at their respective MIC-MA doses of 0.9 g/L, 220 mg/L, and 12 mg/L, metabolic activities of all cells were completely inhibited throughout 24-h exposure. Furthermore, lower combined concentration of above three intracanal disinfectants can elicit equivalent metabolism-inhibiting effect with that of solo use of each one at the MIC-MA dose.

Thus, binary combined use of disinfectants can outperform their solo use in controlling infection of *E. faecalis* and reducing side effects. In conclusion, the MIC-MA derived from D_2O-probed single-cell Raman microspectroscopy is a promising approach in quantitatively assessing disinfectants' antimicrobial efficacy via metabolic activity.



Highlight

- D_2O-probed single-cell Raman spectroscopy on RACS-Seq enables comparative analysis of metabolic inhibition dynamics in *Enterococcus faecalis* by tracking C–D ratio variations under both single and combined disinfectant treatments.

- Liu Y, *et al*. Single-cell Raman microspectroscopy-based assessment of three intracanal disinfectants' effect on *Enterococcus faecalis*. *J Raman Spectro*. 2022, 53(5): 902-910. https://doi.org/10.1002/jrs.6311

Tracking heavy water (D_2O) incorporation for identifying and sorting active microbial cells

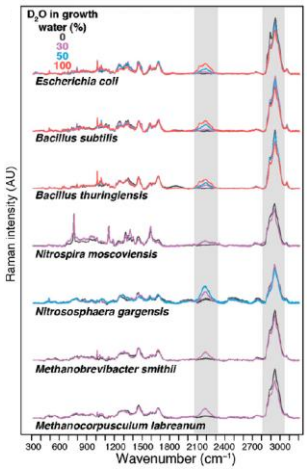
David Berry[a], Esther Mader[a], Tae Kwon Lee[a], Dagmar Woebken[a], Yun Wang[b,c], Di Zhu[c], Marton Palatinszky[a], Arno Schintlmeister[a,d], Markus C. Schmid[a], Buck T. Hanson[a], Naama Shterzer[e], Itzhak Mizrahi[e], Isabella Rauch[f], Thomas Decker[f], Thomas Bocklitz[g], Jürgen Popp[g,h], Christopher M. Gibson[i], Patrick W. Fowler[i], Wei E. Huang[c,j], and Michael Wagner[a,d,1]

Abstract

Microbial communities are essential to the function of virtually all ecosystems and eukaryotes, including humans. However, it is still a major challenge to identify microbial cells active under natural conditions in complex systems.

In this study, we developed a new method to identify and sort active microbes on the single-cell level in complex samples using stable isotope probing with heavy water (D_2O) combined with Raman microspectroscopy. Incorporation of D_2O-derived D into the biomass of autotrophic and heterotrophic bacteria and archaea could be unambiguously detected via C-D signature peaks in single-cell Raman spectra, and the obtained labeling pattern was confirmed by nanoscale resolution secondary ion MS. In fast-growing *Escherichia coli* cells, label detection was already possible after 20 min. For functional analyses of microbial communities, the detection of D incorporation from D_2O in individual microbial cells via Raman microspectroscopy can be directly combined with FISH for the identification of active microbes.

Applying this approach to mouse cecal microbiota revealed that the host-compound foragers *Akkermansia muciniphila* and *Bacteroides acidifaciens* exhibited distinctive response patterns to amendments of mucin and sugars. By Raman-based cell sorting of active (deuterated) cells with optical tweezers and subsequent multiple displacement amplification and DNA sequencing, novel cecal microbes stimulated by mucin and/or glucosamine were identified, demonstrating the potential of the nondestructive D_2O-Raman approach for targeted sorting of microbial cells with defined functional properties for single-cell genomics.



Highlight

- A nondestructive D_2O-Raman method, enabled by RACS-Seq and combining stable isotope probing, Raman microspectroscopy, and optical tweezers, allows for the identification, sorting, and subsequent genomic analysis of active microbial cells in complex microbiota.

- Berry D, *et al*. Tracking heavy water (D_2O) incorporation for identifying and sorting active microbial cells. *Proc Natl Acad Sci U S A*. 2015, 112(2): E194-203.
https://doi.org/10.1073/pnas.1420406112

techsupport@ecytebio.com | www.ecytebio.com
eCyte, Inc., 880 W. Maude Ave, Sunnyvale, CA 94085-2920