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Short communication

# Automated and integrated ultrahigh throughput screening for industrial strain enabled by acoustic-droplet-ejection mass spectrometry

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Synthetic biology provides unprecedented opportunities to tackle critical issues including climate change and sustainable development by constructing microbial cell factories to produce industrially valuable biochemicals, biofuels, and biomaterials using renewable biomass resources [1], where strain screening for specific metabolic traits is a critical step. Rapid, accurate, and simultaneous quantification of multiple metabolites is critical for multi-perspective strain performance evaluation and strain screening, and a generalized method or platform will also reduce method development time to speed up the screening process. Numerous methods have been developed for screening including biochemical assays, fluorometric techniques, and analytic chemistry methods. All of these approaches face challenges in achieving a balance between sensitivity, specificity, reproducibility, versatility, scalability and cost-effectiveness. Biochemical assays reply on specific enzymatic reactions or interactions, while fluorometric screening requires specific fluorescent biomarkers tailored to certain metabolites. Development of these methods can be intricate and time-consuming. Analytical chemistry methods, one the other hand, are powerful in their precision and versatility, but typically involve sophisticated equipment and procedures such as mass spectrometry (MS) or chromatography. Furthermore, they often involve complex sample preparation and lengthy analysis times, making them less practical for high-throughput screening applications.

In the present work, we have developed an innovative platform designated ultrahigh throughput pretreatment and analysis (UTPA) system by seamlessly integrating specialized automated equipment and acoustic droplet ejection (ADE)-open port interface (OPI)-MS [2,3]. The pioneering platform marks for the first instance of achieving high-speed metabolite pretreatment and analysis in industrial strain screening at an unprecedented rate of seconds per sample. It delivers both high accuracy and precision, significantly enhancing the efficiency of the screening process.

The UTPA platform consists of two modules: a customized sample pretreatment system (module A) and an ADE-OPI-MS system (module B). In module A, the consumable hotel, handler workstation, and positive pressure filter are interconnected using a wrench within the workstation. In module B, Echo® MS (SCIEX, Singapore) is employed for ADE-OPI-MS system, which consists of an ADE transducer, an OPI system (including OPI head, transfer capillary, and spray capillary) and a triple quadrupole mass spectrometer equipped with an OptiFlow electrospray ionization source. A 30-inch servo shuttle robot facilitates microplates transfer between the workstation, centrifuge, and Echo® MS (Fig. S1). SAMI software and other tools listed in Table S1 are used to facilitate seamless inline integration of modules A and B, achieving a high level of automation. The workflow of module A comprises five sequential steps: broth separation, sample dilution, transfer, centrifugation, and vortexing. The entire pretreatment for 384-well

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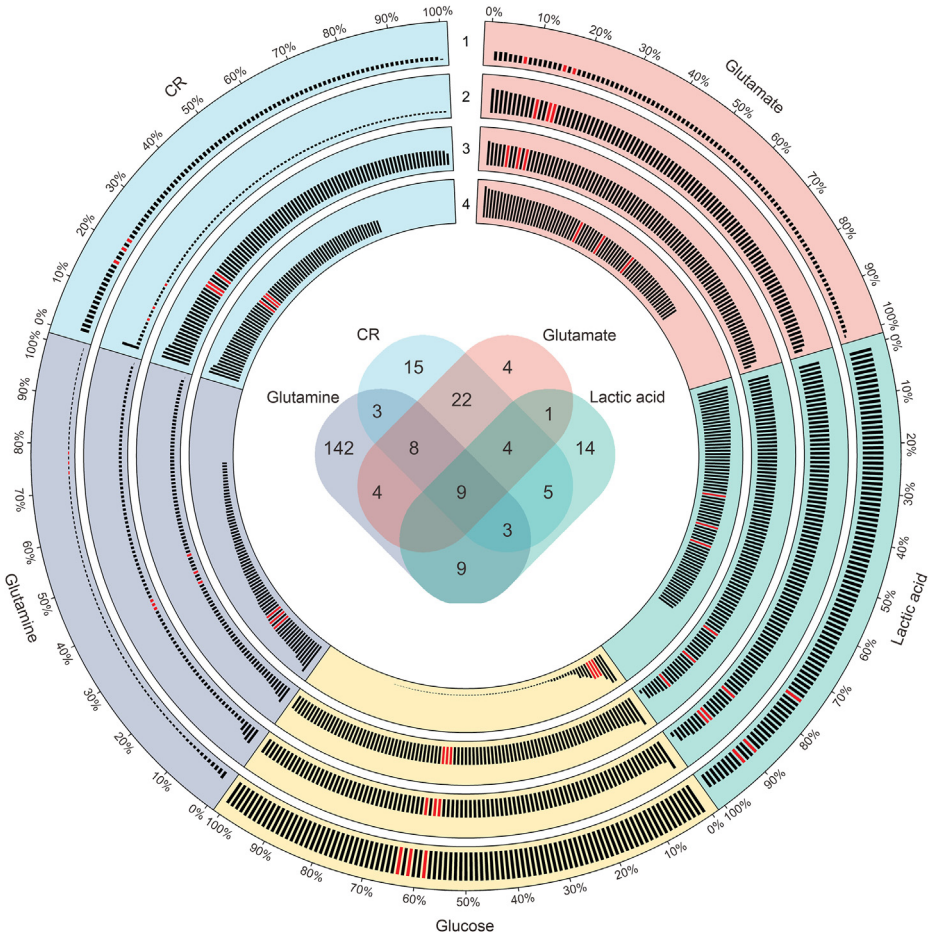


**Fig. 1.** Glutamate hyperproducer strains screening using ultrahigh-throughput pretreatment and analysis (UTPA) platform. 348 test samples (black columns) and 12 control strain samples (red columns) were inoculated to four 96-well plates for subculture. Tracks 1–4 represented the 96-well plates 1–4. In each track, the test samples were arranged according to the level of the analyte's contents, and percentages were used for rapid observation of positive rates. The central Venn diagram shows intersections of positive strains screened using different criteria. CR: glucose-glutamate conversion rate.

plate requires less than 20 min. In module B, the sample acquisition time varies between 1 and 3 s, depending on the set delay time for each sample. This translates to a total duration of 6.4–19.2 min for a single injection of a 384-well plate. To synchronize the timestamp of acoustic unit during data acquisition, a control well (A1, a sample well) is used at both the start and end of each run, adding lag time of approximately 1 min to the overall acquisition duration. Consequently, the complete run time for module B ranges around 7–20 min. The UTPA platform efficiently enables the pretreatment and analysis of 384 strain fermentation samples within 27–40 min, averaging approximately 5 s per sample. The process is fully automated by a robotic arm without the need for human intervention. Enzymatic reaction samples, benefiting from a simplified preparation protocol, are analyzed in about 3 s per sample. Compared to manual processing, the UTPA platform significantly reduces the time and costs, boasting a maximum capacity of approximately 17,000 to 28,000 samples per day.

We showcased the capability of our UTPA platform by screening glutamate hyperproducer strains of *Corynebacterium glutamicum* across six fermentation media (Table S2). By tracking four representative metabolites (glutamate as the target products, glutamine as the potential interference and byproduct, lactic acid as the major byproduct, and glucose as the fermentation substrates), the strain performance was evaluated from multiple levels.

Firstly, the ADE-OPI-MS conditions were optimized to enhance detection sensitivity and minimize interferences. The characteristic multiple reaction monitoring (MRM) ion transitions for four metabolites and their stable isotope internal standards (SIISs) were optimized (Table S3). The optimal carrier fluid was identified as a mixture of 50% methanol and 50% acetonitrile with 2 mM ammonium fluoride, flowing at a rate of 550 μL/min (Figs. S2 and S3). An additional 0.5-s pause between consecutive samples was selected to achieve for sufficient baseline separation (Fig. S4). The base analysis time was set to 1 s per sample. Additionally, 8 MRM transitions were split into 2 injections to ensure sufficient dwell time, resulting in a total analysis time of 20.2 min ($384 \times 2 \times 1.5$ s $+ 1$ min) for 384 samples. Next, this optimized bioanalytical method was validated in compliance with the principles of Bioanalytical Method Validation Guidance for Industry [4]. The material and methods were shown in the Supplementary data. The system suitability, linearity, sensitivity, carryover, accuracy, and precision were evaluated and acceptable (Tables S4–S6 and Fig. S5). The comparison of absolute matrix effects revealed significant variations across four metabolites (Fig. S6A). With increasing dilution, the matrix factor was improved and eventually reached 1 at a 200-fold dilution (Fig. S6B). However, it was still recommended to use the SIISs to eliminate the influence of matrix effects (Table S7).

In the present study, we focused on screening the glutamate-producing strain library derived from SCg5 [5], which was previously stored in our laboratory. This library was created by using diverse engineering procedures, including random chemical

mutagenesis, random enzymatic mutagenesis, and targeted genetic modification. A total of 384 samples including 348 test strains randomly selected from library, 12 control strains harboring a backbone plasmid, 6 calibrators, and 18 quality control samples (three concentration levels were summarized in Table S8) were together assembled into a screen panel, processed, and analyzed. Of the four examined metabolites, glutamate was identified as the primary indicator for screening. Among the 348 test strains, 52 (14.9%) showed a higher glutamate concentration than the control. However, it is not scientifically rigorous to evaluate the production capacity of a strain solely based on the glutamate yield. For industrial strains, substrate-product conversion rate is also a critical metric, because the substrate, typically glucose, occupies more 50% of the production cost for bulky chemical production such as glutamate. In our case, the glucose-glutamate conversion rate (CR) was defined as the ratio of glutamate produced to the glucose consumed. Lactic acid and glutamine were the major biological byproducts. Therefore, to maximize glutamate concentration and CR value while minimizing the production of lactic acid and glutamine, we screened out nine strains (Fig. 1), reducing the positivity rate from 14.9% (52/348) using only glutamate to 2.6% (9/348). The use of multi-indicator screening can significantly reduce the positivity rate and decrease the workload and time required for further validation using larger-scale bioreactors.

In summary, the UTPA platform showcased remarkable efficiency by rapidly screening 348 test strains in 40 min. In comparison to the conventional liquid chromatography (LC)-MS method, the UTPA platform significantly reduced the screening time from two days to just 40 min, accelerating the screening process by an impressive 72 times. Our UTPA platform therefore provides the unprecedented capability of ultrahigh throughput strain screening with high accuracy and precision, opening the gate for rapid cell factory construction and thus having significant potential to future biofoundries. It is expected to be widely adopted in various scenarios that necessitate the rapid collection of extensive biological data, such as in the fields of synthetic biology and systems biology.

## CRediT author statement

**Zhidan Zhang:** Methodology, Formal analysis, Writing - Original draft preparation, Visualization, Investigation; **Chao Zhang:** Methodology, Writing - Reviewing and Editing; **Xu Zhang:** Methodology, Investigation, Data curation, Formal analysis; **Jiuzhou Chen:** Investigation, Resources, Writing - Original draft preparation; **Ningyun Cai** and **Shasha Zhong:** Methodology, Investigation, Resources; **Zhibo Han:** Validation, Formal analysis; **Yan Zhu:** Writing - Reviewing and Editing; **Ping Zheng:** Writing - Reviewing and Editing, Supervision, Conceptualization; **Jibin Sun:** Supervision, Conceptualization, Funding acquisition, Writing - Reviewing and Editing; **Changxiao Liu:** Writing - Reviewing and Editing, Conceptualization.

## Declaration of competing interest

The authors declare that there are no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2024.02.003.

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