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REVIEW PAPER

Quantitative Methods for Metabolite Analysis in Metabolic Engineering

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Abstract We present a brief overview of metabolic engineering, depicting the necessity of exploiting microorganisms for obtaining desired metabolites and the difficulty of metabolic pathway optimization under numerous conditions. The advantages, limitations, and examples of conventional quantitative analytical methods that focus on accuracy but have low throughput rates are presented. We have also described *in vivo* analytical methods with high-throughput rates, which indirectly compare the yield of the reporter protein. Additionally, we have explained a few considerations for engineering *in vivo* analytical methods. This review also highlights the current challenges faced by the analytical methods in the metabolic engineering.

Keywords: metabolic engineering, metabolite analysis, throughput, metabolite-responsive transcription factor, riboswitch

1. Introduction

A metabolite can be a substrate, intermediate, or end-product of a metabolic pathway. Metabolites can be classified into two categories: primary and secondary metabolites. Primary metabolites are essential in metabolic pathways and for cell growth, development, and reproduction. In contrast, secondary metabolites are not essential for the survival of an organism and collectively include antibiotics [1] and other physiologically active substances [2]. The chemical synthesis of these biomolecules is complicated, and extraction from natural sources is inefficient because of low yields [3]. For example, the amount of taxol obtained by sacrificing a 100-year-old Pacific yew tree is approximately 300 mg, which is equivalent to a single dose [4].

Metabolic engineering [5], an alternative to conventional methods, introduces biosynthetic metabolic pathways into genetically tractable heterologous hosts, such as *Escherichia coli* [6,7] and *Saccharomyces cerevisiae* [8], to produce target metabolites. This strategy is more eco-friendly [9] than consuming natural resources. Additionally, scalable fermentation processes are expected to yield fine chemicals [10,11], fuels [6,12], and pharmaceuticals [7,13] at a low cost. For the efficient production of target metabolites, a diverse range of engineering techniques, such as redesigning metabolic pathways in microorganisms [14], selection [15] and ratio adjustment [16] of the participating enzymes, and improving the rate-limiting enzyme activity [17], is required. Hence, research has been focused on quantitatively analyzing the production rate and yield of metabolites to optimize the production process.

Metabolites are typically small molecules, and various methods can be used for their quantification. In metabolic engineering, evolution libraries that produce metabolites of interest generally generate 10^7-10^9 mutations per week, and the ideal throughput rates can detect all these mutations [18]. The analytical methods used thus far include high-performance liquid chromatography (HPLC) [19] and liquid chromatography-mass spectrometry (LC-MS) [20], which can accurately quantify the target metabolites in a sample mixture. The yield of metabolites is quantified at the end of the analysis, which requires substantial time and workforce. Thus, the throughput rate is extremely low (10^3/day) [21]. For example, the high-throughput rate of HPLC validation for nimesulide, a type of analgesic, is 1 sample/min [22]. Simultaneous analysis of the real-time production rate is required owing to the diverse conditions tested during metabolic pathway design and the consequently high number

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of metabolites in each sample. Optimizing conditions using conventional analytical methods is difficult. Thus, various analytical methods have been proposed to increase the throughput rates, and research on improved analytical methods is currently in progress.

In this review, we introduce various methods for quantitatively analyzing metabolites and propose directions for future improvements.

2. Conventional *In Vitro* Metabolite Analytical Methods

The overriding goal of conventional quantitative analytical methods is accurate quantification of the target metabolite concentration in each sample, with a primary focus on "accuracy" and "resolution." However, the disadvantage of these methods is the long time required for sample preparation, optimization of the analysis environment, and individual analysis due to limited sample injection, sample analysis, and data processing, resulting in low throughput rates.

2.1. High-performance liquid chromatography

Over the past 50 years, HPLC has become a prominent technology in analytical chemistry. It is popular in laboratories worldwide for its ability to separate and quantify different substances in a mixture [23]. HPLC is ordinarily used for the isolation or quantification of specific components in complex clinical or biological samples and purification after pharmaceutical production. HPLC relies on the difference in movement velocity in the column depending on the degree of interaction of the analyte with the mobile and stationary phase, that is, the solvent and adsorbent material (Fig. 1).

The main advantage of HPLC is that accurate and highly reproducible quantitative analysis is possible for a wide range of analytes [24], from food [25,26] to environmental samples [27,28].

However, HPLC is not an optimal solution. First, not all components elute from the column (Fig. 1) because HPLC is based on the difference in the polarity of samples; therefore, separating samples with similar polarities is challenging [29,30]. In extreme cases, the analyte is irreversibly adsorbed onto the column, thereby precluding its detection [31]. Furthermore, the operation of the HPLC equipment, which is a collection of devices, requires a significant workforce, as highly skilled technicians continuously monitor and execute the purification per the protocol. Finally, optimization is time-consuming because modules, columns, and mobile phases are sample-specific [32], and the throughput rate is critically low due to individual analyte injection [33]. Additionally, because a large amount of organic solvent is required in reversed-phase HPLC [34], the operating cost with respect to the number of samples that can be analyzed is high.

To address the limitations of HPLC, ultra-performance liquid chromatography (UPLC) was developed using the same principle but with improved performance [35-37]. The analyte is passed through a column with a filler particle size of 2 μm [35] or less at a pressure of at least 15,000 psi [36,37]. According to the Van Deemter equation, the efficiency of chromatography is inversely proportional to the particle size [38]. Therefore, smaller particle size results in better resolution, increased efficiency, improved sensitivity, better separation efficiency per unit column length, and increased flow rate, resulting in an increased resolution. For the same analyte, if the size of the column packing is halved, the analysis time is halved, and the separation efficiency is doubled [39]. However, the reduced filler particle size makes packing the fine particles in the column difficult and also the pressure drop required for the

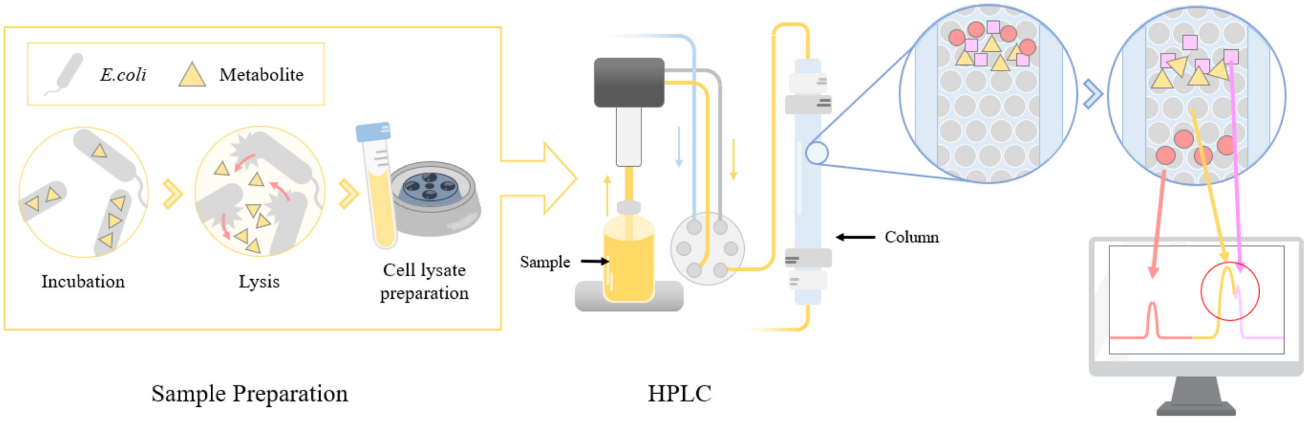

Fig. 1. Schematic diagram of HPLC. HPLC: high-performance liquid chromatography.

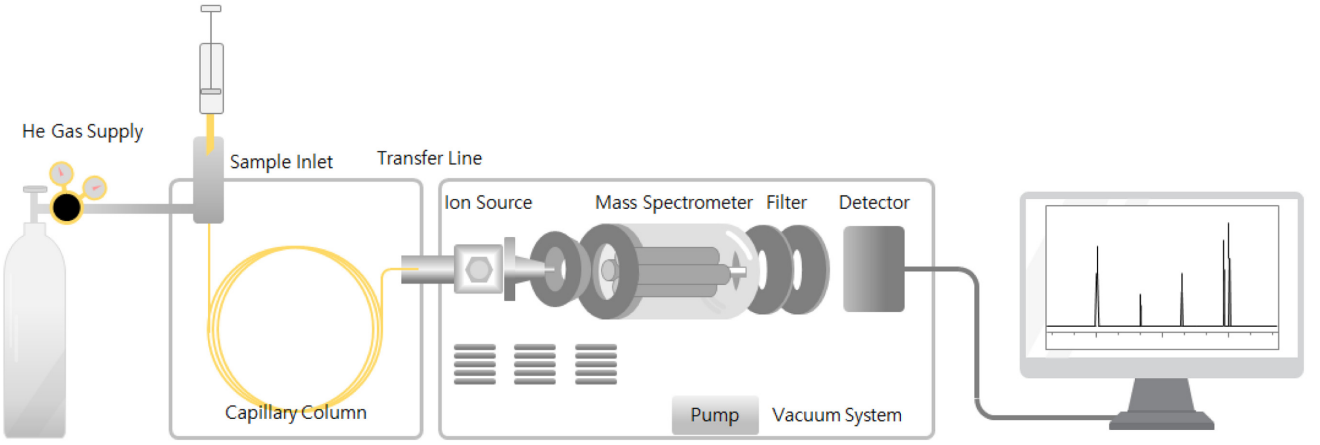

Fig. 2. Schematic diagram of GC-MS. GC-MS: gas chromatography-mass spectrometry.

solvent to pass through the column excessively high [40,41]. Additionally, despite the increased throughput, the throughput rate of UPLC does not exceed 1,000 samples per day, as approximately 100 samples could be analyzed in 8 h [39].

Examples of metabolites quantified using HPLC include salvianic acid A [42], a potential therapeutic agent for cardiovascular disease; L-tyrosine [43], a precursor of levodopa, a treatment drug for Parkinson's disease; and cinnamaldehyde [44], a type of nematicide. Additionally, UPLC was used to quantify levodopa [45].

2.2. Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is used to separate volatile compounds from mixtures without decomposition [46]. GC-MS consists of two units: GC and MS. First, the components dissolved in the solvent are vaporized. The mobile phase mainly used in GC is an inert gas such as helium, which prevents the interaction between the mobile phase and the analyte. The analyte is then inserted into a preheated column. The retention time of the components depends on the relative affinity of the analyte with the column. Each component, separated by a difference in retention time, enters the MS. Free electrons are emitted from the filaments of the downstream MS, which collide with the gaseous molecules discharged from the GC, causing electron ionization where the molecules form their fragments [47]. The ionized fragments decomposed by the MS form a mass spectrum using the mass-to-charge ratio and are quantified by calculating the peak area (Fig. 2).

GC used in isolation results in similar retention times, thereby rendering it impossible to distinguish between other components eluted together. Using MS alone has a similar pattern of ionized fragments in the mass spectrum. LC-MS can allow precise detection owing to the improbability of different molecules being detected simultaneously by both devices [48]. Additionally, GC-MS requires only a small amount of sample for injection and is extremely sensitive even for detecting metabolites at the picogram level [49,50]. It is easy to perform, automated, and provides reproducible results.

However, the sample detection by GC is limited to volatile and thermally stable compounds. Generally, the injector temperature of GC is > 250°C, which can thermally decompose some metabolites [51-53]. Moreover, GC-MS cannot process multiple samples simultaneously, and a single analysis requires a minimum of 15 min. It operates at a throughput rate of 90 analyses per day for metabolite analysis [54].

GC-MS was used to quantify terpenoids, which are widely used as perfumes, pesticides, and antibacterial agents, and taxadiene, an intermediate of paclitaxel, for cancer treatment [55].

2.3. Liquid chromatography-mass spectrometry

LC-MS is an analytical method that combines the physical resolution of liquid chromatography with the mass analysis capability of a mass spectrometer (Fig. 3). Compared to GS-MS, the development of LC-MS was slow because the selection of an appropriate interface between the HPLC eluent and the mass spectrometer was difficult. The standard techniques used for LC-MS applications are electrospray ionization and atmospheric pressure chemical ionization. Since ionization occurs at atmospheric pressure, these sources are commonly referred to as atmospheric pressure ionization (API) sources [56]. The column effluent nebulized by the API forms droplets that are dissolved and become an ion source for mass spectrometry. The frequency of the ion measured by mass spectrometry is presented as a mass spectrum, and the peak area of analysis is quantified.

LC-MS is advantageous as it is more sensitive than HPLC. For certain drugs, LC-MS has exhibited a detection

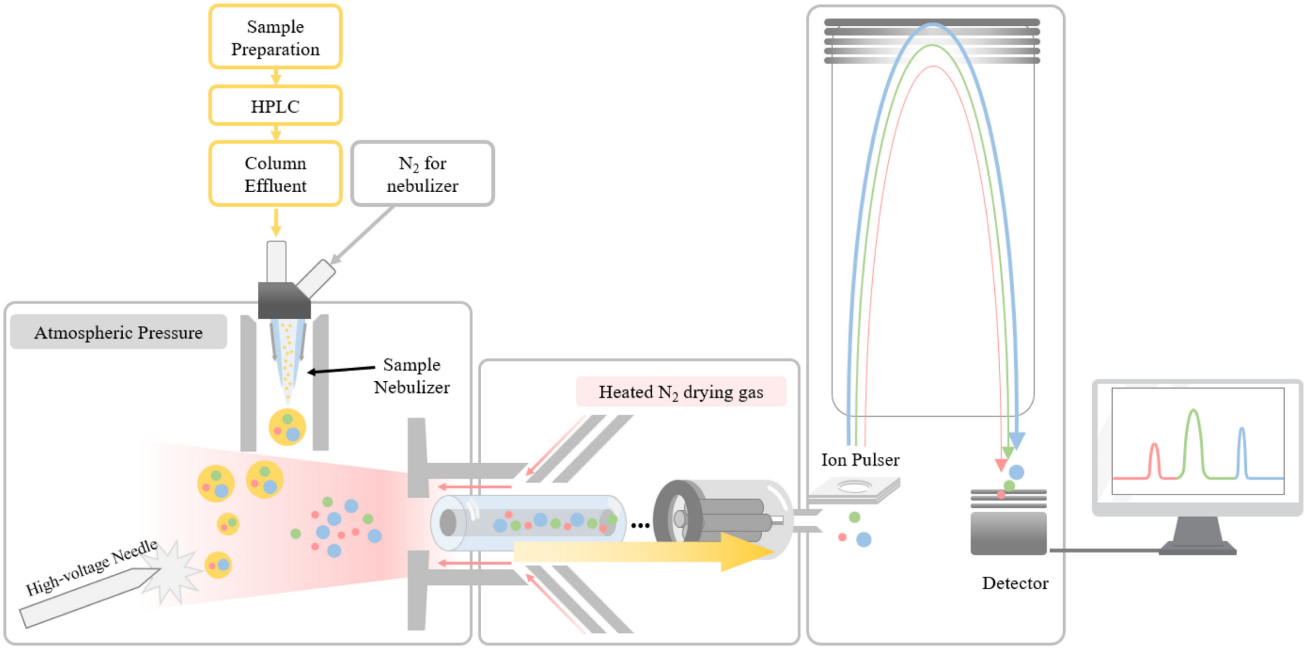

Fig. 3. Schematic diagram of LC-MS. LC-MS: liquid chromatography-mass spectrometry, HPLC: high-performance liquid chromatography.

limit approximately 50 times lower than that of HPLC [57]. In complex mixtures, LC-MS can also be used to identify and quantify sub-ppm units [58,59].

However, similar to HPLC, LC-MS requires specialized technicians to operate the equipment and analyze the data [60-62]. Nonetheless, the range of identifiable compounds may be limited by the comparison with the reference spectrum since the experimental conditions influence the spectrum obtained in LC-MS. Moreover, samples that are not readily available or cannot be obtained in large quantities must be handled carefully, as mass spectrometers consume samples for detection, unlike HPLC, where samples can be collected. Finally, there are limitations to buffer selection [63,64]. This is because using a buffer with low volatility seriously interferes with ion generation [65], as contamination of the ion source due to continuous deposition causes degradation of the LC/MS performance. Thus, a non-volatile phosphate buffer [66], widely used for HPLC, cannot be used for LC-MS.

LC-MS was used to quantify scopolamine, nicotine, and berberine [67], a plant alkaloid with pharmacological activity, and 6-deoxyerythronolide B [68], a precursor of the antibiotic erythromycin.

2.4. Capillary electrophoresis

Capillary electrophoresis (CE) is an electrokinetic separation method that uses a microfluidic channel and capillary with a sub-millimeter diameter [69]. In CE, the analyte moves with electrophoretic mobility through the electrolyte solution under the influence of an electric field (Fig. 4A). Application of high voltage to the capillary filled with the electrolyte results in the formation of a double cation layer. A strong electroosmotic flow is produced [70], and the analytes move rapidly to the cathode (Fig. 4B). Each analyte is separated by the difference in their electrophoretic mobility, detected just before the capillary outlet, and these differences are converted into an electropherogram.

Because the capillary size is usually minuscule with an inner diameter of 20-100 μm [71], organic solvent consumption is minimized, and Joule heat dissipation is highly efficient [72]. Therefore, high voltages of up to 30,000 V can be applied [73]. Using high voltage is advantageous as it allows for fast separation and smaller band broadening; therefore, high sensitivity and sharp peaks can be obtained.

In addition, simultaneous analyses of samples are possible using instruments with capillary arrays to achieve higher throughput rate [74]. Capillary array electrophoresis with 16 or 96 capillaries is used for medium-to-high throughput capillary DNA sequencing and can be spatially arranged to connect the capillary inlet directly to a 96-well plate.

However, the sensitivity of the detector is relatively low. Detection devices used for CE generally use UV or UV-vis absorbance, and the path length of the detection cell (~50 μm) is shorter than that of a conventional UV cell (~1 cm). According to the Beer–Lambert law, the sensitivity of a detector is proportional to the path length of the cell [75]. Therefore, the resolution may be partially lost if the detection path length is increased to improve the sensitivity.

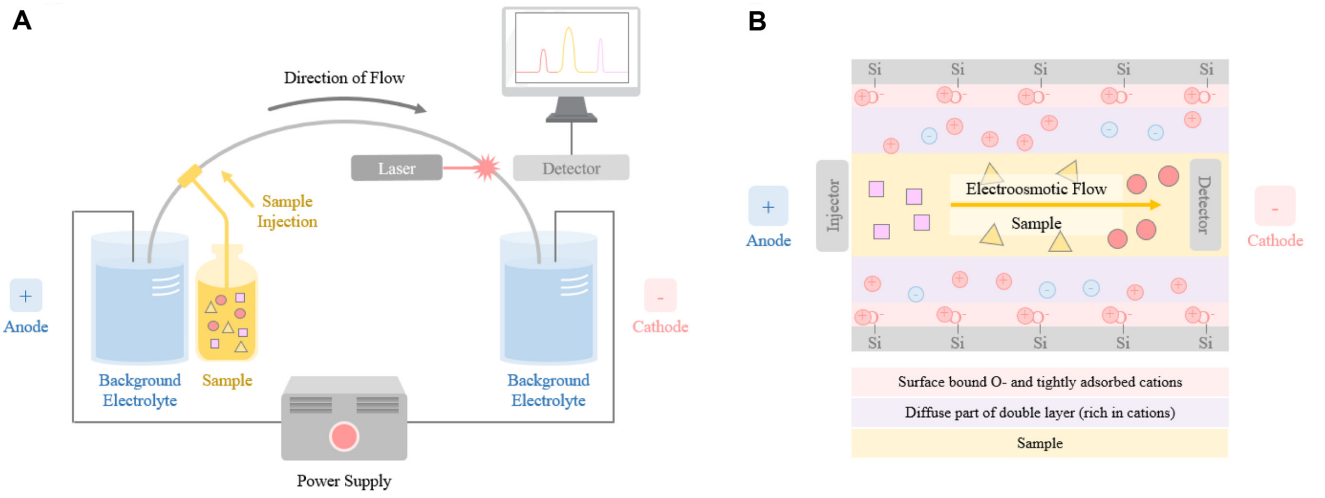

Fig. 4. (A) Schematic diagram of CE. (B) Formation of electroosmotic flow and separation of single component. CE: capillary electrophoresis.

CE was used to quantify lovastatin [76], a drug used to lower cholesterol and reduce the risk of cardiovascular diseases, and succinic acid [77], which is used as a food additive.

2.5. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a technique that observes the local magnetic field around the nucleus of an atom (Fig. 5). The principle of NMR is that many nuclei have a spin, and all nuclei are electrically charged. Exposure to an external magnetic field excites nuclei from the ground to a higher energy state owing to the energy transfer that occurs at radio frequencies [78]. Magnetic field changes around the atom change the resonance frequency, which reveals details about the electrical structure and functional groups present in the

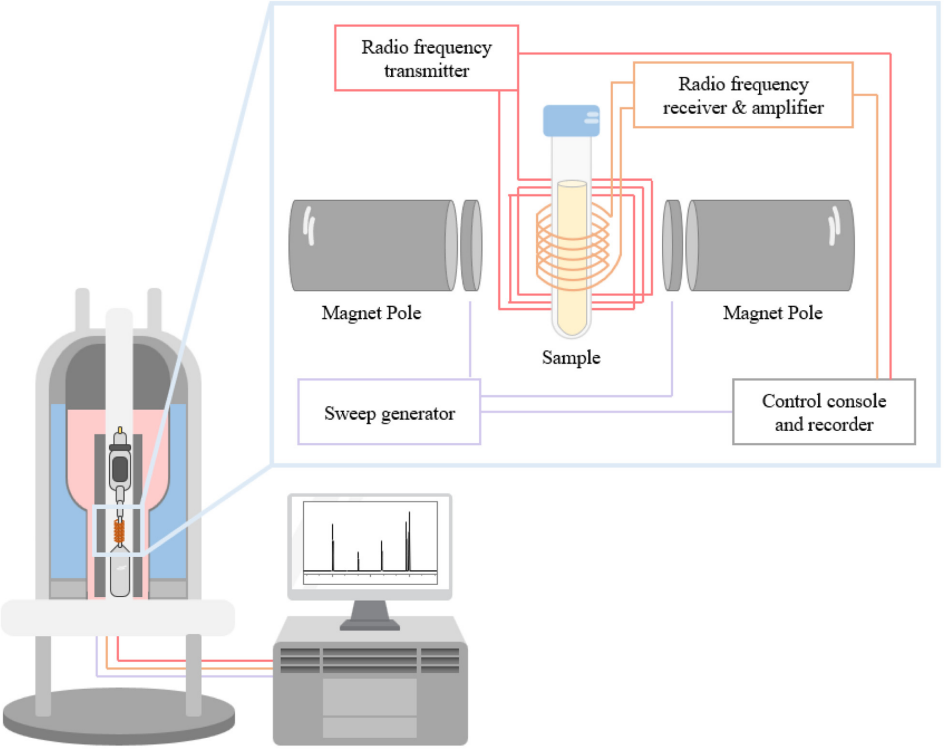

Fig. 5. Schematic diagram of NMR. NMR: nuclear magnetic resonance.

molecules.

As NMR spectra exhibit different patterns for each component and have excellent resolution [79], they are beneficial for predicting structures of small molecules, such as metabolites. In particular, stereoisomers with the same molecular composition but different three-dimensional arrangements can be distinguished [80].

However, one of the most significant drawbacks of NMR is that the amount of sample required for analysis is relatively large compared with that required in other methods (typically 10-50 mg) [81,82], implying that many of the materials required for the quantitative analysis of metabolites are consumed. Furthermore, NMR is an expensive method compared to other analytical methods. The resolution of NMR spectroscopy directly depends on the strength of the magnetic field generated by a superconducting magnet cooled by liquid helium [83]. Inexpensive permanent magnets can be used as a substitute for superconducting magnets; however, their resolution is relatively poor [84]. Finally, the throughput rate of this method is extremely low (< 10^2/day) [85]. Since parallel and simultaneous analyses are not possible, NMR can only analyze individual samples.

Quantitative NMR spectroscopy (qNMR), one of the applied techniques of NMR, is an analytical method based on the principle that the signal intensity of the resonance line is proportional to the resonant nuclei (spin) [86]. In 1963, Hollis [87] performed the first qNMR on a commercially available analgesic sample, quantifying aspirin with a deviation of 1.1%. In metabolic engineering, qNMR has been applied to quantify surfactin [88], a potent surfactant used as an antibiotic.

3. *In Vivo* Analytical Methods Developed to Improve Throughput Rates

The conventional *in vitro* quantitative analytical methods can quantify metabolites with relative accuracy; however, the time consumed to analyze samples with many variables is substantial owing to low throughput rates. Therefore, more advanced metabolite quantification methods aim to increase "throughput rates." Additionally, these methods can be utilized "*in vivo*".

3.1. Metabolite-responsive transcription factor-based biosensor

A transcription factor (TF) or sequence-specific DNA-binding factor is a protein that regulates the rate of messenger RNA (mRNA) transcription by recognizing and binding to

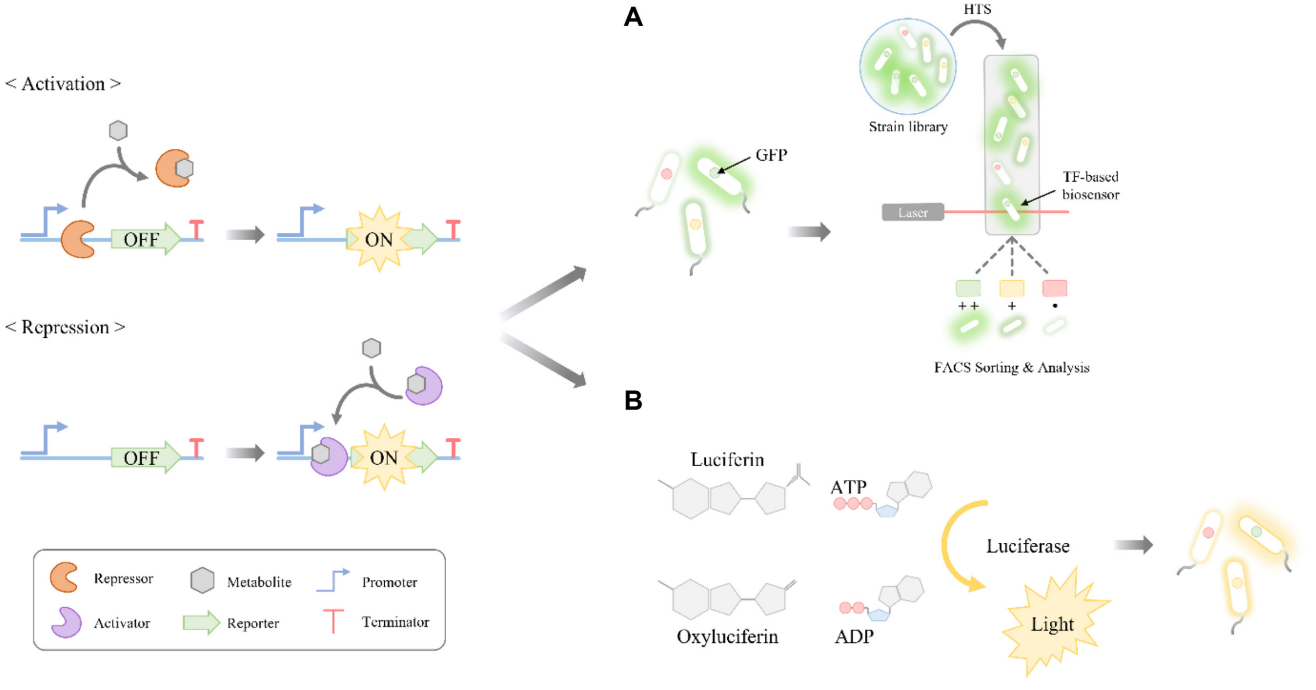

Fig. 6. Schematic diagram of MRTF-based biosensor. Repressor MRTF binds upstream of the target gene to inhibit RNA polymerase. Metabolite activates the expression of the target gene by removing the repressor MRTF. Activator MRTF bound to metabolite accelerates transcription. The reporter protein is mainly (A) the fluorescent protein or (B) luciferase. MRTF: metabolite-responsive transcription factor, GFP: green fluorescent protein, TF: transcription factor, FACS: fluorescence-activated cell sorting, HTS: high-throughput screening, ATP: adenosine triphosphate, ADP: adenosine disphosphate.

a specific DNA sequence (Fig. 6). A TF alone or combined with other proteins promotes or represses the transcription rate of a specific gene by RNA polymerase. A TF has at least one DNA-binding domain, which allows it to attach to a specific sequence of DNA adjacent to the gene it regulates. Interactions with biomolecules or environmental stress can induce conformational changes in the TF, which can change the binding affinity of RNA polymerase with the regulated promoter [89,90].

A metabolite-responsive transcription factor (MRTF) is a TF that responds to natural metabolites [91]. MRTFs are widely used in many organisms; for example, in *E. coli*, one-third of all TFs are MRTFs [92]. The structure of an MRTF used as a biosensor in a heterologous host is divided into two modules: a detection module for the interaction between metabolites and the TF and a control module for the TF to regulate the expression of a target gene. The control modules interacting with RNA polymerases are usually designed to drive the transcription of reporter genes, the output of which can be easily measured optically. The fluorescence output from the accumulation of fluorescent protein (preferred as a reporter protein) indirectly reflects the concentration of the target chemical. However, it requires an excitation light for fluorescence and emits some noise. Additionally, fluorescent molecules are sensitive to reaction conditions such as pH and cations, which can result in the loss of the fluorescence signal. In *S. cerevisiae*, green fluorescent protein (GFP), as a transcriptional output of MRTF-based biosensor for even-chain C8–C12 fatty acids detection, was 13-to 17-fold higher than that in the off state [93]. Another alternative reporter, luciferase, oxidizes substrates like ATP and furimazine, resulting in a bioluminescent signal. The three most widely used luciferases are derived from *Photinus pyralis*, *Renilla reniformis*, and *Oplophorus gracilirostris*. They have different molecular weights (62, 36, 19 kDa, respectively) and emission wavelengths (560, 480, and 460 nm, respectively); therefore, they can be applied to construct a multi-signal system [91]. Compared to fluorescent proteins, these are stable and have a low background signal but are substrate-dependent. Luciferase was used in the metabolic engineering of *Yarrowia lipolytica*, producing violacein [94], a natural antibiotic pigment. Because both reporter proteins are expressed continuously [95], the accumulation of signal molecules or enzymatic reactions can result in signal amplification [96].

In conventional methods, the yield of the target metabolite is directly quantified *in vitro* only after complete production. However, using MRTF-based biosensors, researchers have access to valuable information such as real-time monitoring of metabolite levels. Through this, a deep understanding of the kinetics and regulatory mechanisms constituting the metabolic pathway leads to the design of an efficient strategy within the metabolic pathway to increase the production titer of the metabolite. Consequently, it can alleviate the bottleneck of the design-build-test cycle of metabolic engineering [97].

Furthermore, a considerably high-throughput rate can be obtained by combining an MRTF-based biosensor with fluorescence-activated cell sorting (FACS). FACS, a type of flow cytometry, can categorize a mixture of cells individually into several types based on the fluorescence properties of each cell. FACS enables rapid and quantitative recording of fluorescence signals from individual cells, allowing indirect comparison of target metabolite production with MRTF-based biosensors expressing fluorescent proteins, such as GFP. An MRTF biosensor-based FACS screening could identify a mutant library with 10 million mutants in 30 min to identify L-lysine-producing *E. coli* strains [98].

A major limitation of MRTF-based biosensors is that naturally occurring TFs do not bind to all metabolites. To solve this problem, the sensing profile can be extended by the point mutation of the binding pocket in natural TF [99]. The well-known LacI TF that responds to the disaccharide allolactose has also been engineered to respond to non-metabolized gentiobiose, fucose, lactitol, and sucralose [100]. In another example, *Acinetobacter* TF PoBR exhibited specificity with a detection limit of 2 μM by converting native effector 4-hydroxybenzoate to p-nitrophenol [101]. These could be achieved using Rosetta, a computational enzyme design tool; however, it cannot be applied to allostery [102,103]. Alternatively, random amino acids can be introduced in the active site of the TF (a total of 20^N cases for N amino acid sequences) [104]. However, compared with nucleic acid-based selection (total of 4^N cases for N nucleic acid sequences), the variation is substantially large to confirm entire cases. In conclusion, TF engineering requires many trial-and-error methods because it involves nucleic acid sequence manipulation and complex protein engineering [105], rendering it inherently challenging.

3.2. Small molecule binding riboswitch

Aptamers are single-stranded oligonucleotides that can selectively bind to targets such as proteins, peptides, carbohydrates, and small molecules [106]. Aptamers were selected from a random DNA/RNA library *in vitro* through a process called systemic evolution of ligands by exponential enrichment (SELEX), exhibiting a high affinity for the target [107]. The selected aptamer formed secondary and tertiary structures with a binding domain for the target material.

Aptamers are comparable to antibodies because the binding specificity between an aptamer and the target is comparable to the binding of an antibody to an antigen [108,109]. Unlike antibodies composed of proteins (~180

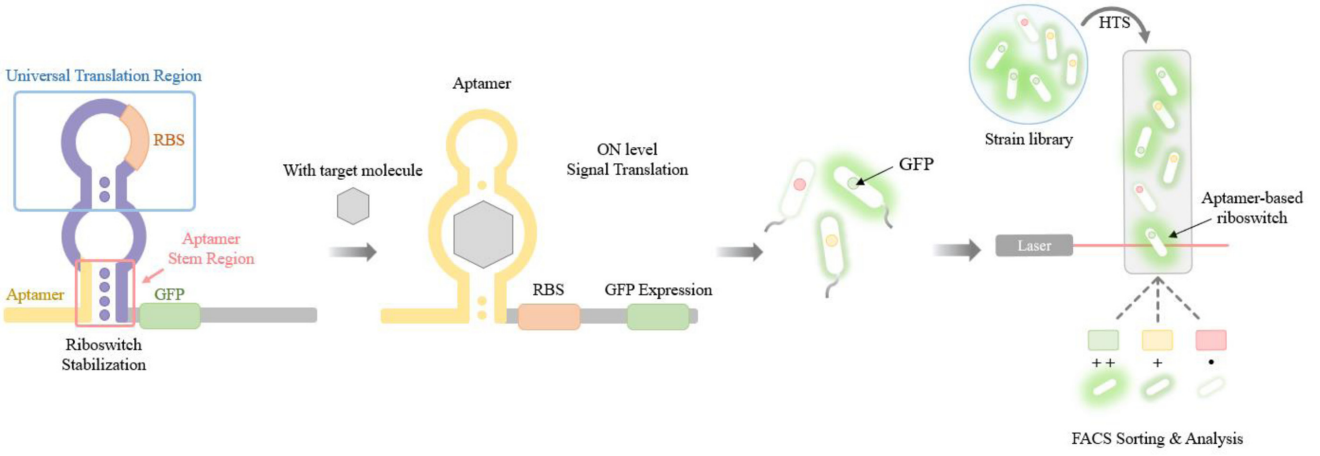

Fig. 7. Schematic diagram of small molecule binding riboswitch. Binding of metabolites induces the expression of the target gene by changing the structure of the riboswitch. RBS: ribosome binding site, GFP: green fluorescent protein, FACS: fluorescence-activated cell sorting, HTS: high-throughput screening.

kDa), aptamers are composed of nucleic acids and are relatively small (~30 kDa). Aptamers can recognize a smaller target (~60 Da) than antibodies (~600 Da) and can bind to various targets, unlike antibodies which are limited to an immunogenic molecule. Therefore, in metabolic engineering, aptamers are preferred over antibodies for quantitative analysis of small molecule metabolites (~1,500 Da) [110,111]. Additionally, aptamers can be readily chemically synthesized, and chemical modification is also relatively tolerant. Therefore, aptamers are an inexpensive and stable solution.

The riboswitch constitutes a part of mRNA and controls the protein expression from mRNA, depending on whether it binds to a target [112]. In general, the structure of a riboswitch is composed of an aptamer sensor domain that detects a target metabolite, a ribosome binding site (RBS), and an expression domain encoding a protein for signaling [113]. In the absence of the target metabolite, the sequence around the RBS forms a stem structure, the RBS is blocked, and the protein is not expressed. However, in the presence of the target metabolite, it binds to the aptamer and changes the secondary structure of the riboswitch, which leads to RBS exposure and allows the protein to be expressed (Fig. 7). It is possible to indirectly quantify and compare the concentration of metabolites through the signal difference in the ON-OFF state, that is, by controlling the dynamic range [114].

This metabolite-binding riboswitch can be used for high-throughput *in vivo* screening [115]. An engineered riboswitch, which responds to naringenin, a type of flavonoid, combined with FACS enabled screening of 100,000 cells within 22 h [116].

Nevertheless, discovering aptamers using SELEX for small-molecule metabolites is challenging primarily because the surface fixation moiety for aptamer discovery is limited [117]. In small molecules, this moiety may be one of the few epitopes for aptamer binding and can no longer be used as a binding site when used for conjugation. In addition, if chemical modifications are applied for immobilization, the aptamer may be screened for variants other than the original target, thereby reducing binding specificity. Finally, in both immobilization methods, the selected aptamer may have a binding affinity for the solid matrix itself [118].

To overcome this, a method called Capture SELEX has been proposed [119-122] in which the N30 DNA library, and not the target material, is immobilized on a streptavidin-agarose column through a short biotinylated complementary oligonucleotide. Target molecules are passed through the column and aptamer candidates with an affinity to a free target are displaced from the column by forming a structure. Then they are collected and amplified using polymerase chain reaction (PCR) for selection in the next round. Since the PCR product is double-stranded DNA (dsDNA), biotinylated antisense primers should be adapted in the PCR to convert them into single strands. After immobilizing dsDNA on a streptavidin-agarose column, hydrogen bonds between the strands are broken using sodium hydroxide, allowing the single-stranded sense DNA to pass through the column. Counter-selection can be employed for analogs to enhance the selectivity of the aptamer. An aptamer with high binding affinity and a dissociation constant of 61 nM to cannabinoids was discovered using this process [123].

Additionally, a high-affinity aptamer does not necessarily produce an effective riboswitch. Typically, a relatively high aptamer affinity results in the operation of the riboswitch in response to minor changes in the metabolite concentration; thus, the operational range is narrowed. In one study, an aptamer was selected with a higher dissociation constant,

Table 1. Comparison of properties of improved metabolite analytical methods

Feature	MRTF	Small molecule binding aptamer
Library size (number of sequences: n)	Large (20^n)	Small (4^n)
Conformational change	Abundant	Minimal
Engineering	Complicated	Simple

MRTF: metabolite-responsive transcription factor.

that is, weak binding, for *in vivo* quantitation of high concentrations of L-tryptophan, which is industrially important and is primarily produced in microbial cell factories [124].

Aptamer selection is not all riboswitch engineering. Using NUPACK [125], a type of nucleic acid secondary structure prediction software, a riboflavin-binding riboswitch was engineered to stabilize the ON-state conformation, enhancing riboflavin yield in *Bacillus subtilis* [126].

The RNA-based riboswitches with aptamers have a smaller library than protein-based sensors with TF. Additionally, screening target substances using aptamers is relatively straightforward (Table 1). In addition, RNA-based sensors can directly interact with the target molecules and are less dependent on the expression of protein effectors, thereby reducing the unnecessary metabolic burden on cells [113].

4. Conclusion

A prerequisite for developing a cell factory that efficiently produces metabolites is screening various conditions involved in metabolic pathways. The conditions to be considered for metabolic pathway optimization include enzyme origin, ratio, and the number of enzymes involved in the pathway. Therefore, high-throughput analyses are required to efficiently determine the optimal conditions.

In this review, we have discussed the advantages and disadvantages of different methods used to detect and quantify metabolites during metabolic engineering. The existing methods can accurately analyze samples after reaction completion but with low throughput rates. To overcome this, a method for promptly converting the concentration of target material into easily measurable luminescence and fluorescence signals has been proposed. Individually including cells containing protein-based (TF) or RNA-based (riboswitch) biosensors in the microdroplet and categorizing the generated signal using FACS can aid in processing several samples within a short time [127]. Nevertheless, challenges remain in monitoring the production rate in real-time while maintaining throughput rates. If both conditions are satisfied in the quantitative metabolite analysis, the evaluation of the phenotype of the strain from different perspectives will be possible.

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Ethical Statements

The authors declare no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

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