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Optimized sampling protocol for mass spectrometry-based metabolomics in *Streptomyces*

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Abstract

In quantitative metabolomics studies, the most crucial step was arresting snapshots of all interesting metabolites. However, the procedure customized for *Streptomyces* was so rare that most studies consulted the procedure from other bacteria even yeast, leading to inaccurate and unreliable metabolomics analysis. In this study, a base solution (acetone: ethanol = 1:1, mol/mol) was added to a quenching solution to keep the integrity of the cell membrane. Based on the molar transition energy (E_T) of the organic solvents, five solutions were used to carry out the quenching procedures. These were acetone, isoamylol, propanol, methanol, and 60% (v/v) methanol. To the best of our knowledge, this is the first report which has utilized a quenching solution with E_T values. Three procedures were also adopted for extraction. These were boiling, freezing–thawing, and grinding ethanol. Following the analysis of the mass balance, amino acids, organic acids, phosphate sugars, and sugar alcohols were measured using gas chromatography with an isotope dilution mass spectrometry. It was found that using isoamylol with a base solution (5:1, v/v) as a quenching solution and that freezing–thawing in liquid nitrogen within 50% (v/v) methanol as an extracting procedure were the best pairing for the quantitative metabolomics of *Streptomyces* ZYJ-6, and resulted in average recoveries of close to 100%. The concentration of intracellular metabolites obtained from this new quenching solution was between two and ten times higher than that from 60% (v/v) methanol, which until now has been the most commonly used solution. Our findings are the first systematic quantitative metabolomics tools for *Streptomyces* ZYJ-6 and, therefore, will be important references for research in fields such as ^{13}C based metabolic flux analysis, multi-omic research and genome-scale metabolic model establishment, as well as for other *Streptomyces*.

Keywords: Quantitative metabolomics, *Streptomyces*, Quenching, Leakage, Extraction

Introduction

Streptomyces, which belong to prokaryotes but show filamentous growth, are the most noteworthy producers of novel medicines for resisting various diseases including cancers, immunological diseases, and infections caused by bacteria, fungi, viruses and parasites. In the 1950s and 60 s, following the golden era of antibiotics discovery, around 70 to 80% of antibiotics that treated bacteria and fungi were isolated from diverse *Streptomyces* (Berdy 2005). At present, *Streptomyces* continue to attract attention due to the need to discover new medicines to break the drug resistance of pathogenic micro-organism (Hwang et al. 2014). As a result of this significant history, plenty of researchers have focused on *Streptomyces*. However, many of their mechanisms still need to be explained. As a result, a comprehensive investigation of *Streptomyces* is now indispensable.

Many authors have described the relevant mechanisms of *Streptomyces* using genomics, transcriptomics, proteomics, and so on, but there are hardly any metabolomics analyses which illuminate the metabolic mechanism of *Streptomyces*. Metabolomics is the best tool for understanding cell metabolism and the relationship between genotypes and phenotypes. Specifically,

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given improvements in mass spectrometry techniques, the field of metabolomics has experienced significant growth (Carnicer et al. 2012). However, while there have been many improvements in metabolomics, perfecting the procedure used to prepare biological samples is still a challenge. Many metabolites not only have a short turnover time which are just seconds or less, but they also have different physicochemical properties and extremely low intracellular concentrations (de Jonge et al. 2012; Douma et al. 2010). This means that in quantitative metabolomics studies, the key step is defining snapshots of all interesting metabolites. To this end, rapid sampling, fast quenching, and effective extraction procedures are crucial for ensuring the real status of intracellular metabolites. Due to the seconds or sub-seconds turnover time, keeping rapid sampling at a time of less than one second is reasonable. When quenching, it is essential that first, enzymes are inactivated immediately to ensure no loss or (inter)conversion of metabolites; second, that cells are kept intact to avert intracellular leakage; and third, that extracellular diffusion and exposure time are kept as short as possible to guard against the noxious effects of organic solvents. During the process of extraction, thorough, non-selective, and non-destructive operations are necessary.

Notably, Smart et al. developed an analytical platform for the metabolomic analysis of microbial cells (that is, yeast, filamentous fungi, and bacteria) using methylchloroformate (MCF) derivatization followed by GC–MS (Cheng et al. 2013). They demonstrated that using cold glycerol-saline solution as a quenching agent could stop the cell metabolism of different microbial cells (such as *Streptomyces coelicolor*) (Smart et al. 2010) while minimizing the leakage of intracellular compounds into the quenching solution, thus allowing the reliable separation of intracellular and extracellular metabolites (Villas-Bôas and Bruheim 2007). Specifically, cold glycerol saline appeared to have potential as a quenching solution for an accurate intracellular metabolite analysis based on MCF derivatization, but it had drawbacks as a result of silylation derivatization (Villas-Bôas and Bruheim 2007), which is the classic and most widely used derivatization procedure for metabolomics analysis by GC–MS (Villas-Boas et al. 2005). In addition, Kassama et al. assessed the extraction procedures of intracellular metabolites for *Streptomyces lividans* and quenched 5 mL of broth in 25 mL of 60% aqueous methanol which contained 10 mM HEPES at − 40 °C (Kassama et al. 2010). Wentzel and colleagues studied intracellular metabolite pool changes in *Streptomyces coelicolor*. In both of these studies, 5 mL of culture sample was withdrawn, filtrated, washed twice with 2.63% (w/v) NaCl solution, and transferred to 25 mL 60% methanol solution at − 23 °C (Wentzel et al. 2012). Unfortunately, the HEPES and NaCl solution had the potential to cause matrix effects on MS-based analysis, meaning that quenching should be finished as quickly as possible to obtain metabolism snapshots. While many efforts have been made to exploit the optimal method, no universal solution has been discovered for each individual micro-organism, meaning that it should be modulated individually.

The sample preparation procedure customized for the study of *Streptomyces* metabolomics is so rare that most researchers have referenced the procedure for other bacteria (Lu et al. 2016; Muhamadali et al. 2015) including yeast (Guo et al. 2015; Cheng et al. 2013). Several representative *Streptomyces* strains along with their procedures for sample pretreatment are listed in Table 1. Sample volumes ranged from 5 to 15 mL. Following sampling, separation was performed using centrifugation or filtration. If washing steps were used, the NaCl solution was the most popular solution for that washing. However, some studies have demonstrated that the washing step would worsen metabolite leakage in bacterial cells (Min et al. 2010). Cold methanol aqueous solution was the most commonly used quenching solution, with temperatures ranging from − 45 to − 20 °C. Most extractions focused on freezing–thawing or grinding in liquid nitrogen with organic solvents such as methanol aqueous solutions.

In this area, the most popular procedure for sample pretreatment was a combination of cold methanol with boiling ethanol. However, it has been robustly shown that cold methanol quenching is always the cause of serious leakage of intracellular metabolites in bacteria (Min et al. 2010; Japelt et al. 2015; Link et al. 2008). In addition, the partial conversion of pyruvate, nucleotides, and phosphate sugars was observed during extraction with boiling ethanol (Winder et al. 2008). In bacteria, the fast filtration method was used and verified to be effective for minimizing the leakage of intracellular metabolites both in Gram-negative and Gram-positive bacteria (Hong et al. 2017; Bolten et al. 2007). This method can, therefore, be used as an alternative to cold methanol quenching. However, there has as yet been no systematic evaluation reported of this sample pretreatment method for *Streptomyces* ZYJ-6.

In this study, aiming to develop comprehensive tools for quantitative intracellular metabolomics of *Streptomyces* ZYJ-6, utilizing quenching methods and extraction procedures which have not been previously reported. By basing quenching solution on their molar transition energy (E_T), we assessed the effects of five different solutions on the leakage of intracellular metabolites of *Streptomyces* ZYJ-6. To our knowledge, this is the first report of optimization quenching solution using molar

Table 1 Typical procedures of sample preparation for *Streptomyces*

Strains	Sampling[a]	Separation[b]	Washing	Quenching[d]	Extraction[e]	Reference strains
Streptomyces albus (Lu et al. 2016)	9 mL sample	Centrifuge	No[c]	Acetonitrile/methanol/0.1% glacial acetate (45:45:10, v/v) to a final volume of 1 mL − 20 °C	Acetonitrile/methanol/0.1% glacial acetate (45:45:10, v/v); 1 mL; − 20 °C	*Escherichia coli*
Streptomyces avermitilis (Guo et al. 2015)	1 sample[g]	Centrifuge	6 mL 0.6% (w/v) NaCl at 4 °C	4 [g] 40% methanol at − 40 °C	1 mL; chloroform: ethanol: water = 2:2:1 (v/v/v), thawed and frozen	*Yeast*
Streptomyces coelicolor (Wentzel et al. 2012b)	5 mL sample	Filtration	5 mL 2.63% (w/v) NaCl	25 mL 60% methanol at − 23 °C	25 mL 60% methanol at − 23 °C, thawed and frozen	Null[f]
Streptomyces hygroscopicus (Wang et al. 2015)	10 mL sample	Centrifuge	10 mL 0.9% (w/v) NaCl at 4 °C	40 mL 60% methanol at − 40 °C	Grinded using liquid nitrogen; 1.0 mL cooled methanol (50% (v/v), − 40 °C); thawed and frozen	*Streptomyces coelicolor*
Streptomyces lividans (Muhamadali et al. 2015)	15 mL sample	Centrifuge	No[c]	30 mL 60% methanol at − 45 °C	0.5 mL 100% methanol at − 45 °C, thawed and frozen	*Escherichia coli*
Streptomyces lydicus (Cheng et al. 2013)			Deionized water	60% methanol at − 40 °C	Grinded using liquid nitrogen; cooled methanol (50% (v/v), − 40 °C)	*Saccharomyces cerevisiae*
Streptomyces tsukubaensis (Xia et al. 2013)	10 mL sample	Filtration	10 mL 0.9% (w/v) NaCl	25 mL 60% methanol at − 40 °C	2.5 mL 50% (v/v) methanol at − 30 °C, thawed and frozen	*Streptomyces coelicolor*
Streptomyces lividans (Kassama et al. 2010)	5 mL sample	Centrifuge	No[c]	25 mL 60% methanol containing 10 mM HEPES at − 40 °C	1 mL 100% methanol at − 40 °C, thawed and frozen	Null[f]

[a] Sample volume for each sampling; [b] separation methods for separating pellet and broth; [c] no washing steps; [d] the ingredient of quenching solution and temperature; [e] the ingredient and operation of extraction and temperature; [f] developing methods for their own strains; [g] fourfold volume quenching solution was added to the sample

transition energy (E_T) and evaluating five quenching solutions and three extraction procedures (see Additional file 1: Table S1) through gas chromatography–isotope dilution mass spectrometry (GC–IDMS) for *Streptomyces*. We believe that our findings will be useful for the quantification of intracellular metabolites in other *Streptomyces*.

Materials and methods

Solvents and chemicals

Chemicals were provided by Shanghai Lingfeng (Chemical Reagent Co., Ltd., China), while analytical grade standards were purchased from Sigma-Aldrich Chemical Co. (USA) and LC–MS-grade solvents were obtained from Fisher Scientific (Thermo Fisher Scientific, USA).

Strain and cultivation

The *Streptomyces* ZYJ-6, a mutant strain with only a single component (FR-008-III) (Zhou et al. 2008), was kindly donated by professor Delin You of Shanghai Jiao Tong University, China.

Aerobic cultivations of *Streptomyces* ZYJ-6 were initiated with glycerol stocks. Spores were harvested from slant culture on SFM medium (2% agar, 2% mannitol, 2% soybean powder, and pH 7.2) after 4-day incubation at 30 °C. Spore suspension was inoculated (10^7 spores per 100 mL^{-1}) in a 500 mL Erlenmeyer flask with 100 mL TSBY medium (3% TSB, 1% yeast extract, 10.3% sucrose, and pH 7.2) and grown for 30 h at 30 °C and 220 rpm. Some 300 mL of mycelia suspension was inoculated in 5 L bioreactor for additional fermentation.

Fermentation culture was carried out in a 5 L turbine-stirred bioreactor (working volume of 3 L) at 30 °C with agitation at 400 rpm. The sterile air was set at 1 vvm through a bottom sparger, maintaining overpressure at 0.05 MPa in chemically defined medium. The medium contained (L^{-1}): glucose 50 g, KH_2PO_4 1.5 g $(NH_4)_2SO_4$ 1.8 g, $EDTANa_2$ 1.8 g, $MgSO_4{\cdot}7H_2O$ 8.6 g, $ZnSO_4{\cdot}7H_2O$ 35.7 mg, $CaCl_2$ 50 mg, $FeSO_4{\cdot}7H_2O$

28.7 mg, $CuSO_4 \cdot 5H_2O$ 42 mg, $MnSO_4 \cdot H_2O$ 9.1 mg, antifoam 0.3%, NaCl 9.0 g as an osmotic pressure regulator, and pH 7.2 was controlled by feeding 10% ammonium hydroxide. The bioreactor with medium was sterilized at 121 °C for 60 min, while the glucose solution was sterilized separately at 110 °C for 40 min.

Preparation of uniformly ^{13}C-labeled cell extracts

Metabolite was accurately quantified by IDMS method (Wu et al. 2005). Extracts from cells grown on uniformly ^{13}C labeled [U-^{13}C] glucose were utilized as internal standards (IS) and added into each sample before extraction. IS was able to trace losses during sample pretreatment (Canelas et al. 2009). This was prepared by cultivating *Pichia pastoris* G/DSEL in 1 L turbine-stirred bioreactor (working volume 0.6 L) and was fed with fully U-^{13}C (20 g/L, 99%, Cambridge Isotope Laboratories, Inc.) labeled glucose as the sole carbon source. Cultivation took 20 h (Hong et al. 2017; Lu et al. 2015). Sampling, quenching, and extraction procedures were carried out as described by Carnicer et al. (2012), although quenching solution was precooled and maintained at − 80 °C.

Sampling

Samples were taken from the bioreactor using a pressure-driven rapid sampling device developed by our laboratory (Additional file 2: Figure S1). This device can adjust sampling time and so control sampling volume. In this study, around 1 mL fermentation broth was withdrawn within 0.2 s during the early stationary phase. This was followed by quick homogenization using a vortex mixer. In addition, samples were divided into fractions based on mass balance requirements (Lameiras et al. 2015). Specifically, this means that IC was intracellular metabolites extracted from cell pellets, that is, the centrifugal sedimentation; EC was extracellular metabolites, which were filtered from the fermentation broth directly; TB was total broth; that is, the broth without centrifugation, which had been subjected to quenching and extraction directly, while QS was the quenching supernatant, which came from the centrifugation mixture of the broth and quenching solution (see intact workflow in Fig. 1).

TB samples were immediately withdrawn into 15 mL centrifuge tubes containing 8 mL of quenching solution precooled at − 30 °C by rapid sampling device in 0.2 s, whereupon they were weighed and provisionally stored at − 30 °C. IC and QS samples were withdrawn with the same manner, although after weighing, they were centrifuged for 1 min at − 13 °C and 6300 RCF. Subsequently, samples were separated to pellet (IC) and supernatant (QS) and provisionally stored in cryostat at − 30 °C. Samples of TB, IC, and QS should then be subjected to

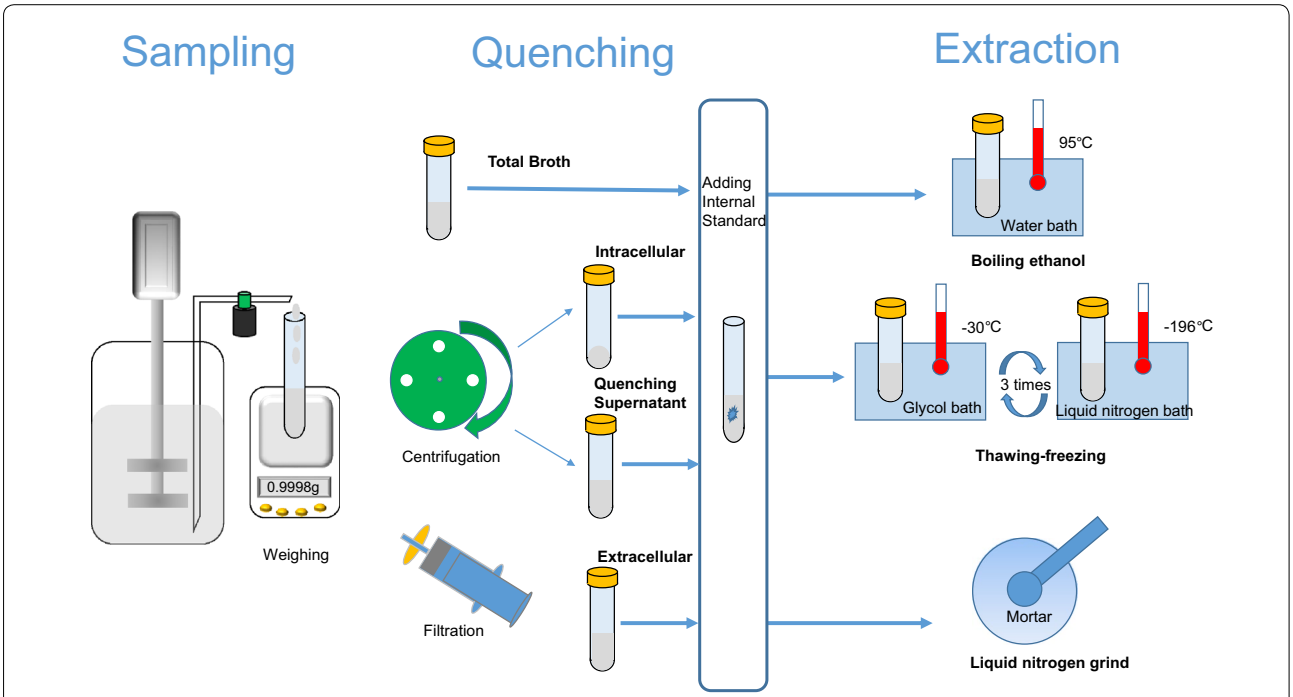

Fig. 1 Schematic representation of rapid sampling, quenching, and extraction procedures for *Streptomyces*. The three extraction procedures were (1) boiling ethanol: 75% (v/v) ethanol in 95 °C 4 min; (2) thawing–freezing in − 30 °C and liquid nitrogen in 50% (v/v) methanol, and (3) grinding liquid nitrogen in 50% (v/v) methanol

extraction of the metabolites. If not, samples should be stored at −80 °C until extraction. EC samples were withdrawn into a filter syringe precooled to −20 °C with 32 g stainless steel beads (2 mm diameter, Shanghai Yalian Hardware Electromechanical Equipment Co., Ltd.), filtrated through a 0.45 μm PVDF filter (RephiLe Bioscience, Ltd.), and attached to a syringe. The filter liquor was then collected into new tubes, snap-frozen in liquid nitrogen, and stored at −80 °C until MS analysis (Mashego et al. 2003).

Quenching and extraction

Quenching solutions in the experimental group were composed in two parts. Part A: Part B = 5:1 (v/v). Part A was the selected solution based on the different molar transition energy (E_T, an empirical quantitative parameter of solvent polarity) (Reichardt and Christian 1979): acetone (E_T = 42.2 kcal mol^{-1}), isoamylol (E_T = 47 kcal mol^{-1}), propanol (E_T = 50.7 kcal mol^{-1}), and methanol (E_T = 55.5 kcal mol^{-1}), respectively. Part B, referred to as the base solution in this study, consisted of acetone (E_T = 42.2 kcal mol^{-1}):ethanol (E_T = 51.9 kcal mol^{-1}) = 1:1 (mol/mol). The quenching solution in the control group was 60% (v/v) methanol aqueous (water: E_T = 63.1 kcal mol^{-1}), as shown in Table 1. The above components were abbreviated as AceB, IsoaB, ProB, MethB, and Meth60, respectively. For example, AceB means that part A was acetone and mixed with part B by 5:1 (v/v). All other abbreviations follow this pattern. Meth60 was a 60% (v/v) methanol aqueous solution without the base solution. In the optimization experiments for quenching solutions, freezing–thawing in liquid nitrogen in 50% (v/v) methanol was used for extraction, following literature summarized in Table 1.

Extraction was optimized using three methods. First, after adding 100 μL IS to the samples (TB, IC, and QS), cell pellets/solutions were suspended/mixed with 30 mL 50% (v/v) methanol at −30 °C. Samples were then subjected to three cycles of freezing in liquid nitrogen for 3 min and thawing at −30 °C on cryostat. The second method followed the same steps as method one, but after being suspended/mixed, the cell pellets/solutions were then ground in a mortar with liquid nitrogen. Third, cell pellets/solutions were suspended/mixed with 30 mL of 75% (v/v) ethanol at 95 °C and the extracted by boiling for 4 min.

Procedures before injection

After extraction, 30–40 mL solution was centrifuged at 3580 RCF for 10 min at −9 °C, while the supernatant was collected in a new tagged centrifuge tube and subjected to concentration. Once its weight was below 0.5 g, the concentrated solution was transferred to a new tube, where milliQ water was added to 0.5 g. After being filtrated with 0.22 μm filter, the 0.5 g concentrated solution was divided into four different aliquots. One and two were 100 μL*2 in gas chromatography (GC) vials, one for measurement of amino acids (AA) and the other for analysis of organic acids (OA), phosphate sugars (PS), and sugar alcohols (SA). The third was 50 μL in liquid chromatography (LC) vials for nucleotides and coenzymes determination, while the fourth was the remainder, stocked at −80 °C for safety. An 80 μL EC sample was drawn into GC vials with 20 μL IS. This resulted in two aliquots: one for AA measurement and the other for analysis of OA, PS, and SA. A 40 μL EC sample was drawn into LC vials with 10 μL IS for determination of nucleotides and coenzymes.

Samples in GC vials were lyophilized and derivatized. For the AA analysis, 75 μL of acetonitrile and 75 μL of *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) were added to the samples. The resultant samples were incubated at 70 °C for 60 min (de Jonge et al. 2011). For analysis of OA, PS, and SA, 50 μL of fresh pyridine methoxamine solution (20 mg mL^{-1}) were added to the GC glass vials. These vials were then incubated at 70 °C for 50 min. After being cooled to room temperature, 80 μL methyl-trimethyl-silyl-trifluoroacetamide (MSTFA) was added, and the samples were then incubated at 70 °C for 50 min (Cipollina et al. 2009). Before injection, samples were centrifuged in 8000 RCF for 1 min, following which the supernatant was transferred to an inner liner.

Metabolites determination

AA, OA, PS, and SA were analyzed using GC–MS with a 7890A GC paired with a 5975C MSD (Agilent, Santa Clara, CA, USA). Conditions for the determination of the metabolites were as defined by de Jonge et al. 2011, with slight modifications to column and temperature gradients. Briefly, 1 μL of sample was injected on a HP5-5% Phenyl Methicone column (30 m × 250 μm internal diameter and 0.25 μm film thickness), using 250 °C for injection in splitless mode. During analysis, the flow velocity of helium was set as 1 mL min^{-1}. The GC column temperature gradient for AA analysis was initially set to 100 °C for 1 min and was then raised by a speed of 10 °C min^{-1} up to 300 °C and held at 300 °C for 10 min. In contrast for OA, PS, and SA, initial temperature was set as 70 °C for 1 min and then increased with a speed of 10 °C min^{-1} up to 300 °C and kept at 300 °C for 10 min. The temperature of transfer line, MS source, and quadrupole were set as 280 °C, 230 °C, and 150 °C, respectively. Electron ionization was always operated with 70 eV. To ensure accurate quantitation, MS was operated in selected ion-monitoring (SIM) mode. This means that the results reported in this study refer to quantitative

targeted metabolomics experiments outside of untargeted metabolomics experiments. The quantification of metabolites was conducted using IDMS (Wordofa et al. 2017), while the unit of metabolites concentration was μmol $gDCW^{-1}$.

Nucleotides and coenzymes were analyzed utilizing an ultra-high-performance liquid chromatography–mass, tandem–mass spectrometry (UHPLC-MS/MS). The Thermal Ultimate 3000 UPLC system was coupled to a Thermal TSQ QUANTUM ULTRA mass spectrum system. Specifically, separation of compounds was conducted by an ion-pairing reverse-phase method on an ACQUITY UPLC BEH C18, 1.7 μm, 150 × 2.1 mm column (Waters Corporation, Milford, MA, US) at 25 °C. The eluent A was 5% acetonitrile aqueous solution with 5 mM dibutylammonium acetate (DBAA), while the eluent B was 84% acetonitrile aqueous solution with 5 mM DBAA. The gradient of eluents A and B was used as defined by Seifar et al. (Seifar et al. 2013, 2009) (Additional file 1: Tables S2 and S3). The injection volume was 2 μL. MS was operated in selected reaction monitoring (SRM) with a negative ion mode. Electrospray ionization parameters were as follows: spray voltage 3000 V, sheath gas pressure 15 arbitrary units, aux gas pressure 10 arbitrary units, ion sweep gas pressure two arbitrary units, capillary temperature 270 °C, and vaporizer temperature 200 °C. Daughter ions, tube lens voltage, and collision energy were optimized individually for each of these compounds (Hong et al. 2017).

Data processing

The raw data of the chromatogram from GC–MS and LC–MS/MS were converted into concentration data using Chemstation from Agilent and Xcalibur from Thermo Fisher, respectively. The calibration curve was defined via IDMS (*X*-axis was the concentration of standard and *Y*-axis was the area ratio of $^{12}C/^{13}C$). Following this, intracellular metabolites data were calculated according to μmol per dry cell weight.

Results and discussion

Batch cultivation of *Streptomyces* ZYJ-6

The strain was grown aerobically in a chemically defined medium. The growth profile is shown in Fig. 2. In order for researchers working in the field of metabolomics to sample rationally, a greater understanding of the physiological parameters of the micro-organism being studied is essential. Three distinct phases were found: during the first 20 h in the lag phase, glucose was consumed slowly; at 24 to 48 h in the exponential phase, glucose consumption increased with vigorous biomass growth; and at 60 h, cells had grown into the plateau, meaning the glucose was almost depleted after activating secondary metabolism. In this study, samples were taken at 60 h, in a stable growth state which ensured that the cell physiology was relatively reproducible.

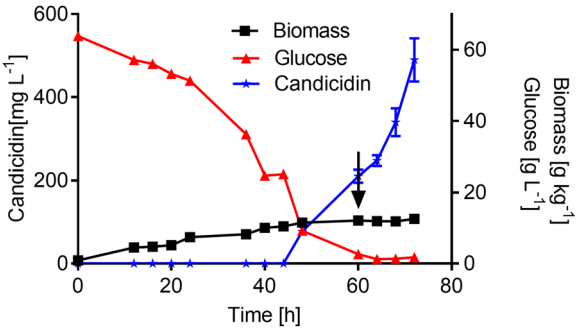

Fig. 2 Growth profiles of *Streptomyces* ZYJ-6 in a chemically defined medium. The arrow represents sampling time at 60 h in a stable growth state, ensuring that the cell physiology was relatively reproducible. Error bars indicate standard deviations of the three independent samples, some of which are hidden under symbols. Diamonds, biomass [g kg^{-1}]; triangles, glucose [g L^{-1}]; pentagrams, candicidin [mg L^{-1}]

Quenching optimization

The suitability of AceB, IsoaB, ProB, MethB, and Meth60 were tested for the metabolomics analysis of *Streptomyces* ZYJ-6, based on the impacts of E_T, mass balance analysis, and prolonged exposure time. The temperature in the quenching experiment process was maintained at below −20 °C to inactivate metabolism. In all optimized approaches, three samples were obtained at a stationary state for biological replicates. In addition, the E_T used in this study ranked from large to small successively were water, methanol, ethanol, propanol, isoamylol, and acetone. It was found that Meth60 possesses higher E_T, while methanol was a small molecule and more liable to permeate the cells. This was probably the main reason for the leakage of intracellular metabolites of bacteria. Therefore, this study also considered the E_T of the base solution. This base solution consisted of acetone and ethanol, the weakest and strongest E_T combination. Ethanol has a larger molecule and stronger E_T, which makes it a good candidate for a base solution for balancing the E_T of the entire quenching solution.

Effect of E_T

The 60% methanol and its derivatives (including some buffers) (Kapoore et al. 2017) have been widely used as a quenching solution for micro-organisms including *Penicillium chrysogenum* (de Jonge et al. 2012), *Aspergillus niger* (Lameiras et al. 2015), *Pichia pastoris* (Carnicer

et al. 2012), and others. Despite this, more studies have verified that 60% methanol was a threat to the integrity of the prokaryotic cell membrane (Japelt et al. 2015; Link et al. 2008). Based on this, we hypothesized that the leakage might be related to the molecular size and E_T value of quenching solutions. We, therefore, evaluated the effects of four combinations of quenching solutions from appropriate molecular sizes, and E_T levels by quenching replicate samples in AceB, IsoaB, ProB, MethB, and Meth60, as well as comparing the concentration of the metabolites measured in the different fractions (Fig. 1).

In this experiment, cryostat temperature was kept at − 30 °C, centrifugation was conducted at − 13 °C, and all other conditions were maintained. The assumptions, results, and conclusions were not suitable for metabolites which existed in both exometabolome and endometabolome in this study. If smaller E_T values resulted in less cell membrane damage, we would expect to find the lowest detectable metabolite levels in the QS fraction (Fig. 3) and highest metabolite levels in the IC (Additional file 2: Figure S2) with the smallest E_T, namely AceB. In Fig. 3, the heatmap was presented following the concentration of metabolites in the QS fraction, which was obtained from the supernatant. Ideally, the concentration in the QS fraction should be as low as possible. However, contrary to our expectations, the AceB solution only performed better than that of MethB, which was the worst in the four combinations tested in this study. As expected, the control group Meth60 gave the biggest red area, meaning that the concentration of metabolites in the QS fraction of Meth60 was the highest, thus suggesting the largest leakage. In contrast, the IsoaB, which had the smaller E_T, showed the best performance with the lowest leakage.

The results in Fig. 3 and Additional file 2: Figure S2 show that in both QS and IC, the IsoaB with smaller E_T was the best quenching solution, while the AceB with the smallest E_T had the poorer performance. It could be speculated that the appropriate E_T for each quenching

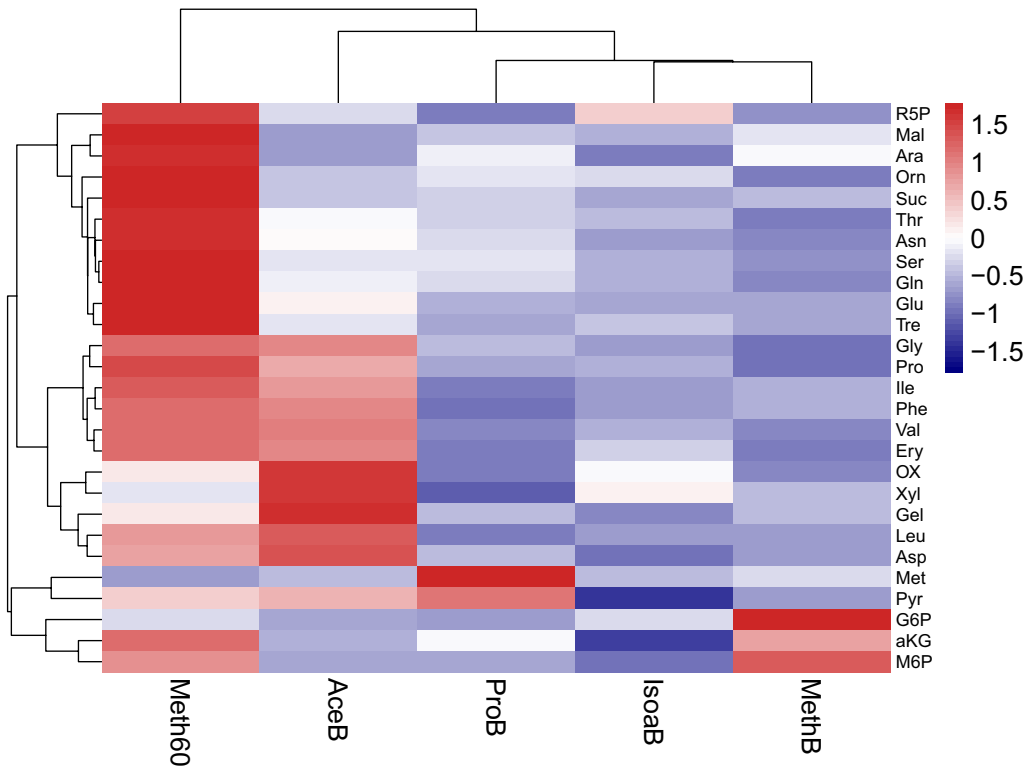

Fig. 3 Hierarchical clustering analysis of the metabolites from the QS fraction. The concentration (μmol $gDCW^{-1}$) of metabolites from the QS fraction was used for clustering analysis. The QS was the quenching supernatant of the centrifugal samples. The QS were attained from Meth60: 60% methanol; AceB: acetone; ProB: propanol; IsoaB: isoamylol; and MethB: methanol with base solution 5:1 (v/v) at − 30 °C, respectively. Base solution was made up of acetone: ethanol = 1:1 (mol/mol). Clustering scaled and distance measure utilized in clustering rows by Euclidean distance. Each row and column indicated an individual metabolite and QS, respectively. *R5P* ribose-5-phosphate, *Mal* malate, *Ara* arabitol, *Orn* ornithine, *Suc* succinate, *Thr* threonine, *Asn* asparaginate, *Ser* serine, *Gln* glutamine, *Glu* glutamate, *Tre* trehalose, *Gly* glycine, *Pro* proline, *Ile* isoleucine; *Phe* phenylalanine, *Val* valine, *Ery* erythritol, *OX* oxalic acid, *Xyl* xylitol, *Gel* glycerol, *Leu* leucine, *Asp* aspartate, *Met* methionine, *Pyr* pyruvate, *G6P* glucose-6-phosphate, *aKG* alpha-ketoglutarate, *M6P* mannose-6-phosphate

solution was needed to avoid leakage. In addition, the MethB (~83% methanol), which was even lower than Meth60 (60% methanol) in the IC areas, serves as a reminder that methanol as a small molecule was threatening for *Streptomyces* ZYJ-6. Thus, results show that as a key parameter in selection of quenching solutions, E_T had its optimal range. In terms of *Streptomyces* ZYJ-6, the best quenching condition was the IsoaB at −30 °C.

Mass balance analysis

The extent of the leakage of metabolites from quenching in AceB, IsoaB, ProB, MethB, and Meth60 was assessed using a quantitative mass balance method (Lameiras et al. 2015). To compare the performance of the five quenching solutions, the ratio of metabolite concentration in IC as well as the difference between the concentrations in TB and EC were calculated for each metabolite from each of the quenching solutions. These ratios can be seen as types of recoveries, namely recovery ratio = IC/(TB − EC) × 100%. It has been stated in the literature (Bolten et al. 2007; Taymaz-Nikerel et al. 2009) that the subtraction procedure is widely accepted for estimating the 'true' intracellular amount.

In Fig. 4, recoveries are represented by boxplots for all of the evaluated metabolites. These were separated into three classes: organic acids (Fig. 4a); phosphorylated sugars (Fig. 4b); and amino acids (Fig. 4c). It is clear that the recoveries which were obtained with AceB as the quenching solution at −30 °C were widely distributed, particularly for organic and amino acids. In contrast, those obtained by IsoaB were closest to 100% and

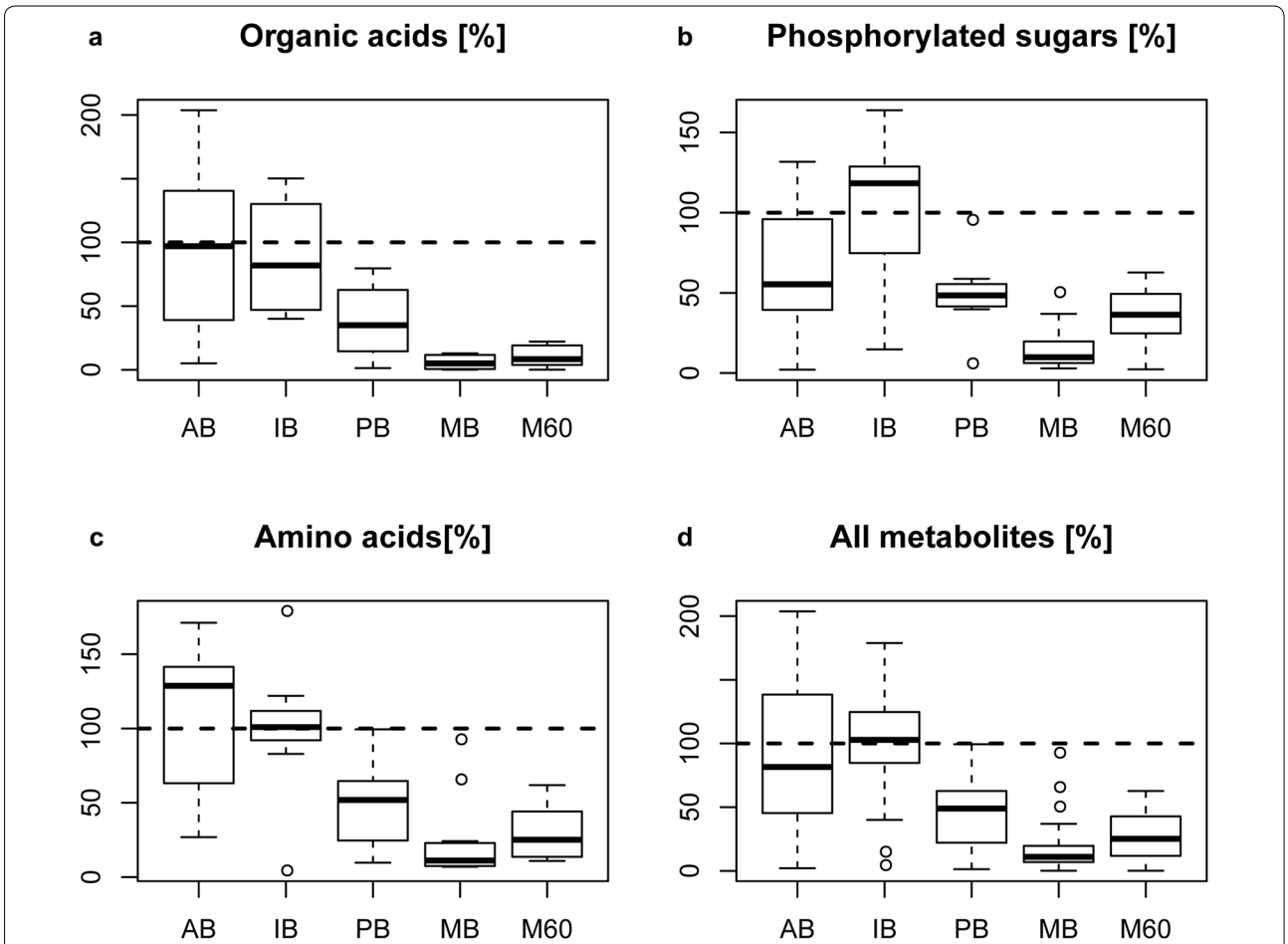

Fig. 4 Box plots of recovery ratios = IC/(TB − EC) × 100%. *IC* intracellular, *TB* total broth and *EC* extracellular for AceB, IsoaB, ProB, MethB, and Meth60. AceB: acetone; IsoaB: isoamylol; ProB: propanol; MethB: methanol with base solution 5:1 (v/v) at −30 °C, respectively; Meth60: 60% methanol at −30 °C. Base solution consisted of acetone: ethanol = 1:1 (mol/mol). Box plots indicated the median, as well as the first and third quartiles. The whiskers indicate the most extreme point from the first and third quartiles within 1.5 times of the distance between the first and third quartiles; points at a greater distance are indicated by circles. The four sections show the results of organic acids (**a** left-top), phosphorylated sugars (**b** right-top), amino acids (**c** left-bottom), and of all metabolites (**d** right-bottom)

amino acids gained perfect recoveries. However, some metabolites were found to exist in both the intracellular and extracellular solutions. These included organic acids which led to fluctuating recoveries. In contrast, even the optimal quenching condition still did not completely prevent leakage. In the case of ProB, almost all metabolites had 50% recoveries. MethB had a poorer performance than Meth60, therefore confirming the conclusions from the E_T element, which again verified that methanol was detrimental to our investigated organism. It was concluded that stronger recoveries were gained when IsoaB was used as the quenching solution at −30 °C for *Streptomyces* ZYJ-6.

Full mass balances for all determinable metabolites in this study are shown in Fig. 5a. For simplicity, metabolite concentrations of all sample fractions are expressed in μmol $gCDW^{-1}$. The concentrations measured in IC should be compared with the TB-EC, and the IC + QS with the TB. It was found that when utilizing AceB as the quenching solution at −30 °C, the obtained data displayed big error bars. These error bars were the same as those applied to all QS parts. This might be caused by the carry-over of sulfates and phosphates (originating from the medium), since high concentrations of these salts interfered with MS-based analysis (van Dam et al. 2002). The comparison between IC and TB-EC is shown in red and gray, respectively. The IC red area in IsoaB was closest to the TB-EC gray area, while the second closest was AceB. Other sections were too low to read, particularly the combination containing methanol, which indicates that metabolite leakage occurred under these conditions. Following this, a comparison of IC + QS and TB were shown first in red and blue and then in black. The IC + QS red and blue area in IsoaB was closest to the TB black area, but we could also observe that the QS blue area in IsoaB was larger than the EC green area in TB-EC. It was shown that there was still some leakage under IsoaB solution at −30 °C for *Streptomyces* ZYJ-6. In fact, metabolites existed in both exometabolome and endometabolome, which could partially explain this phenomenon.

It was concluded that using IsoaB as the quenching solution at −30 °C resulted in the best performance among the five quenching candidates. However, for many metabolites, especially organic acids, this combination was unable to completely prevent leakage.

Impact of prolonged exposure

Much existing research has reported the impact of prolonged exposure in quenching solutions. Koning et al. stopped the leakage of prolonged exposure in the methanol quenching solution by measuring metabolite levels after 30 min of quenching (Koning and Dam 1992). However, results from de Jonge et al. and Canelas et al. demonstrate that prolonging exposure time in quenching methanol solutions aggravates the leakage of intracellular metabolites (de Jonge et al. 2012; Canelas et al. 2008). Our experiment was implemented by keeping samples with IsoaB in the cryostat at −30 °C for +0, +5, or +30 min, respectively before the centrifugation step. We anticipated that the same intracellular levels would be

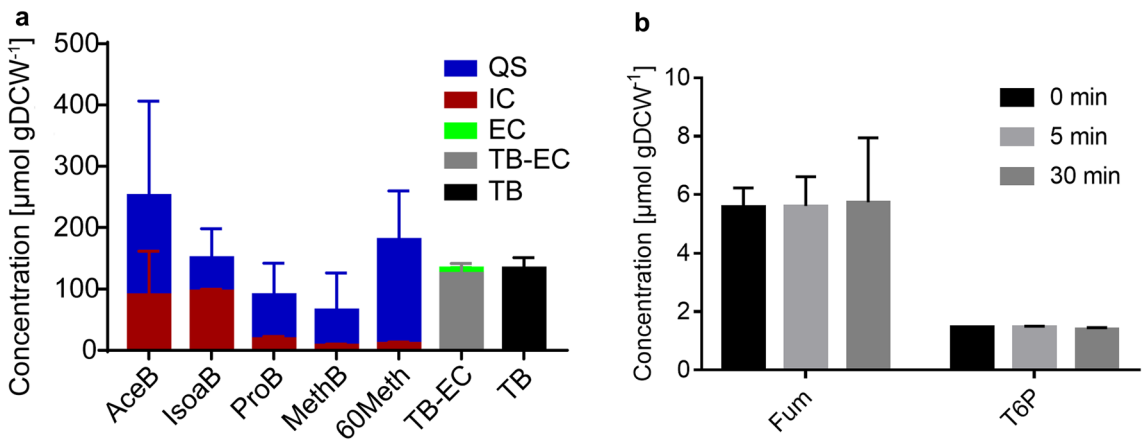

Fig. 5 **a** Full mass balances of all determinable metabolites. Meth60: 60% methanol at −30 °C; AceB: acetone; ProB: propanol; IsoaB: isoamylol; MethB: methanol with base solution 5:1 (v/v) at −30 °C, respectively. The base solution consisted of acetone: ethanol = 1:1 (mol/mol). QS: quenching supernatant; IC: intracellular; TB: total broth; and EC: extracellular. TB-EC: calculated 'true' intracellular metabolites. **b** Effect of prolonged exposure to isoamylol with base solution (5:1 v/v). Based on measured intracellular levels, fumarate (Fum) and trehalose-6-phosphate (T6P) were exemplified. The base solution consisted of acetone: ethanol = 1:1 (mol/mol). Prolonged exposure was achieved by leaving samples at −30 °C + 0, +5, and +30 min before the centrifugation step. Samples were drawn from a culture at 60 h. Data were averages ± standard deviation of the three replicate samples. Each was analyzed in triplicate

found regardless of exposure time if leakage did not take place. As shown in Fig. 5b, fumarate (Fum) and trehalose-6-phosphate (T6P) were typical examples of organic acids (smaller molecule, less polar) and phosphorylated intermediates (larger molecule, more polar), respectively. It is clear that the longer the cells remained in the quenching solution, the larger were the error bars gained, indicating some extent of time-dependent leakage occurring in intracellular metabolites. Interestingly, T6P, which acted as the larger and more polar compound, exhibited a lesser extent of leakage than the smaller and less polar compounds, such as Fum. These results were broadly in agreement with the existing findings (Canelas et al. 2008), suggesting that contact time with the quenching solution should be kept to a minimum, as leakage was not serious in this case.

Evaluation of extraction procedures

In the three extraction tests, we used the same quenching condition of IsoaB as the quenching solution at −30 °C, and three extraction procedures were evaluated. These were first, boiling ethanol (BE), 75% (v/v) ethanol boiling in 95 °C for 4 min; second, thawing–freezing (TF) in cryostat at −30 °C and liquid nitrogen in 50% (v/v) methanol for three cycles; and third, grinding (*G*) liquid nitrogen in 50% (v/v) methanol. The extraction results are shown in Fig. 6a, while the reproducibility of different extraction conditions is shown in Additional file 1: Table S4. Intracellular metabolites were divided into four parts: AA, OA, PS, and SA. However, TF was operated manually, which was time-consuming. These drawbacks would restrict application of TF to global high-throughput analyses.

In contrast, BE could overcome the aforementioned disadvantages to a certain degree. However, some existing studies have reported that several metabolites (pyruvate, nucleotides, and sugar phosphates) (Winder et al. 2008; Maharjan and Ferenci 2003; Villasbôas 2005) were not stable in 95 °C 75% (v/v) ethanol, which could be a fatal defect in the extraction of those metabolites. Grinding (*G*) in liquid nitrogen within 50% (v/v) methanol had some differences across different metabolites, while it is easy to cause experimental human errors and loss of materials during the grinding process. In summary, in the laboratory scale and low-throughput condition, thawing–freezing (TF) in cryostat at −30 °C as well as liquid nitrogen within 50% (v/v) methanol for three cycles was found to be an appropriate extraction procedure for the intracellular extraction of *Streptomyces* ZYJ-6.

Composition of the *Streptomyces* ZYJ-6 metabolomics

In total, 44 intracellular metabolites were found and quantified from AA, OA, PS, SA, nucleotides, and coenzymes, based on IsoaB and Meth60 as the quenching solutions and thawing-freezing (TF) in cryostat at −30 °C and liquid nitrogen in 50% (v/v) methanol for three cycles as the extraction solution. The 44 intracellular metabolites were identified and confirmed using the standards in quantitative targeted metabolomics experiments. Results are displayed in Additional file 1: Table S5. While these metabolites could not represent the entire metabolomics of *Streptomyces* ZYJ-6, they did cover the

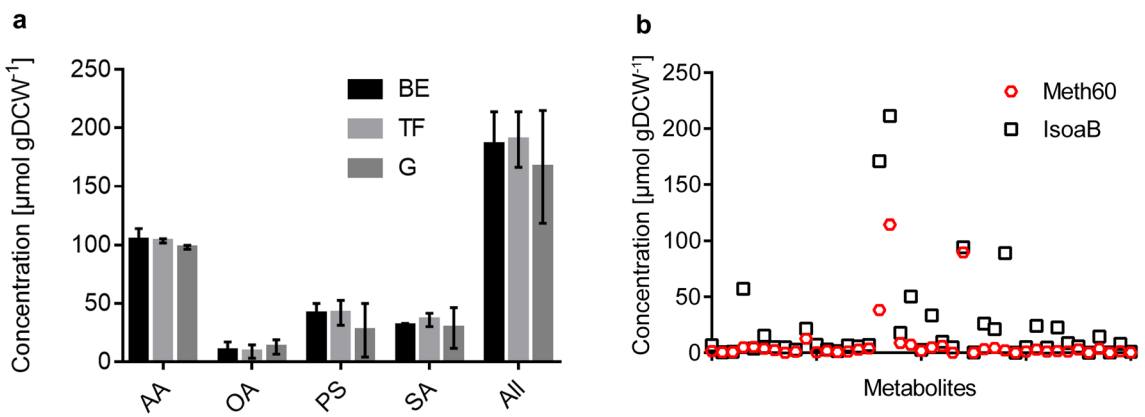

Fig. 6 **a** Extraction performance of the three extraction procedures. BE: boiling ethanol, 75% (v/v) ethanol boiling in 95 °C for 4 min; TF: thawing–freezing in liquid nitrogen and cryostat at −30 °C in 50% (v/v) methanol for three cycles; G: grinding in liquid nitrogen in 50% (v/v) methanol. Metabolites were divided in four parts, which were: *AA* amino acids, *OA* organic acids, *PS* phosphate sugars, *SA* sugar alcohols. All means the summation of these four parts. **b** Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution, and thawing–freezing (TF) in liquid nitrogen and cryostat at −30 °C within 50% (v/v) methanol for three cycles as the extraction procedure. IsoaB: isoamylol with base solution 5:1 (v/v) at −30 °C; and Meth60: 60% methanol at −30 °C, respectively. Base solution consisted of acetone:ethanol = 1:1 (mol/mol). The red polygon represents intracellular metabolites from Meth60 quenching and the black square represents intracellular metabolites from IsoaB. Data were averages ± standard deviation of the three replicate samples, each of which was analyzed in triplicate

most highly abundant metabolites, which played significant roles in central, amino acid and even energy metabolism. The ratio of intracellular metabolites concentrations in IsoaB/Meth60 was calculated and can be seen in Additional file 1: Table S5, which was approximately 2–10, suggesting that the largest concentrations of intracellular metabolites in IsoaB were higher than those in the traditional 60% (v/v) methanol quenching solution. Inspection of Fig. 6b suggests that almost all black squares (intracellular metabolites from IsoaB quenching) were above red polygons (intracellular metabolites from Meth60 quenching), meaning that 60% (v/v) methanol quenching solution may cause severe leakage, and so the IsoaB was the optimal quenching solution for *Streptomyces* ZYJ-6.

Conclusion

First of all, based on the effect of E_T, mass balance analysis, the impact of prolonged exposure, and the quantitative determination of metabolites, we found that isoamylol with a base solution as the quenching solution and thawing–freezing as the extraction procedure were the most reliable, accurate, and reproducible sample pretreatment method to meet our aim. Furthermore, E_T value was an important parameter for choosing the quenching solutions for different micro-organisms. Finally, we demonstrated 44 identified and quantified intracellular metabolites in *Streptomyces* ZYJ-6, which was unprecedented and, therefore, important in the study of metabolomics. Our work is the first to shed light on the tools for quantitative intracellular metabolomics of *Streptomyces* ZYJ-6 and has the potential for significant influence in the relevant fields, such as ^{13}C-based metabolomics flux analysis and multi-omic research as well as genome-scale metabolic model establishment. In addition, this work provided important evidence for research into additional *Streptomyces*.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40643-019-0269-1.

Additional file 1: Table S1. Details of quenching solutions and extraction procedures. Q1–Q5 denotes 5 quenching experiments and E1–E3 denotes 3 extraction procedures. **Table S2.** The gradient and flow rate for the determination of nucleotides. **Table S3.** The gradient and flow rate for the determination of coenzymes. **Table S4.** Reproducibility is assessed by %RSD in different extraction and quenching conditions. **Table S5.** Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution and thawing-freezing (TF) in liquid nitrogen and cryostat at − 30 °C within 50% (v/v) methanol for three cycles as extraction solutions. IsoaB: isoamylol with base solution 5:1 (v/v) at − 30 °C and Meth60: 60% methanol at − 30 °C, respectively. Base solution was made up of acetone: ethanol = 1:1 (mol/mol). Multiples were calculated by IsoaB/Meth60.

Additional file 2: Figure S1. In-house made rapid sampling device and its working procedure (A: diagrammatic sketch; B: front view; C: vertical view). **Figure S2.** Effect of different quenching solutions on the measured intracellular levels. Valine (Val), alpha-ketoglutarate (αKG) and glucose-6-phosphate (G6P) were exampled. Samples were taken from a culture at time = 60 h. AceB: acetone with base solution 5:1 (v/v) at − 30 °C; IsoaB: isoamylol with base solution 5:1 (v/v) at − 30 °C; ProB: propanol with base solution 5:1 (v/v) at − 30 °C; MethB: methanol with base solution 5:1 (v/v) at − 30 °C; Meth60: 60% methanol at − 30 °C, respectively. Base solution was made up of acetone: ethanol = 1:1 (mol/mol). Data were averages ± standard deviation of three replicate samples, each was analyzed in triplicate.

Abbreviations

E_T: molar transition energy; MCF: methyl-chloroformate; GC–MS: gas chromatography–mass spectrometry; IDMS: isotope dilution mass spectrometry; LC–MS: liquid chromatography–mass spectrometry; IC: intracellular metabolites extracted from cell pellets; EC: extracellular metabolites; TB: total broth; QS: quenching supernatant; AceB: acetone:base solution (acetone:ethanol = 1:1, mol/mol) = 5:1 (v/v); IsoaB, isoamylol: base solution (acetone:ethanol = 1:1, mol/mol) = 5:1 (v/v); ProB, propanol: base solution (acetone:ethanol = 1:1, mol/mol) = 5:1 (v/v); MethB methanol: base solution (acetone:ethanol = 1:1, mol/mol) = 5:1 (v/v); Meth60: 60% methanol; IS: internal standard; AA: amino acids; OA: organic acids; PS: phosphate sugars; SA: sugar alcohols; MTBSTFA: *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide; MSTFA: methyl-trimethyl-silyl-trifluoroacetamide; SIM: selected ion monitoring; DCW: dry cell weight; UHPLC-MS/MS: ultra-high-performance liquid chromatography–mass, tandem–mass spectrometry; DBAA: dibutylammonium acetate; SRM: selected reaction monitoring; Fum: fumarate; T6P: trehalose-6-phosphate; BE: boiling ethanol; TF: thawing–freezing; *G*: grinding.

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Authors' contributions

XL, TW, and XS carried out experiments, and XL was the major contributor in writing the manuscript. ZW, XT, YZ, and JC revised manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Additional files to this article can be found online.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Berdy J (2005) Bioactive microbial metabolites. J Antibiot 58(1):1–26
Bolten CJ, Kiefer P, Letisse F, Portais JC, Wittmann C (2007) Sampling for metabolome analysis of microorganisms. Anal Chem 79(10):3843–3849
Canelas AB, Ras C, ten Pierick A, van Dam JC, Heijnen JJ, Van Gulik WM (2008) Leakage-free rapid quenching technique for yeast metabolomics. Metabolomics 4(3):226–239

Canelas AB, ten Pierick A, Ras C, Seifar RM, van Dam JC, van Gulik WM et al (2009) Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolomics. Anal Chem 81(17):7379–7389

Carnicer M, Canelas AB, ten Pierick A, Zeng Z, van Dam J, Albiol J et al (2012) Development of quantitative metabolomics for *Pichia pastoris*. Metabolomics 8(2):284–298

Cheng JS, Cui SF, Ding MZ, Yuan YJ (2013) Insights into the roles of exogenous glutamate and proline in improving streptolydigin production of *Streptomyces lydicus* with metabolomic analysis. J Ind Microbiol Biotechnol 40(11):1303–1314

Cipollina C, ten Pierick A, Canelas AB, Seifar RM, van Maris AJA, van Dam JC et al (2009) A comprehensive method for the quantification of the non-oxidative pentose phosphate pathway intermediates in *Saccharomyces cerevisiae* by GC-IDMS. J Chromatogr B 877(27):3231–3236

de Jonge LP, Buijs NA, ten Pierick A, Deshmukh A, Zhao Z, Kiel JA et al (2011) Scale-down of penicillin production in *Penicillium chrysogenum*. Biotechnol J 6(8):944–958

de Jonge LP, Douma RD, Heijnen JJ, van Gulik WM (2012) Optimization of cold methanol quenching for quantitative metabolomics of *Penicillium chrysogenum*. Metabolomics 8(4):727–735

Douma RD, de Jonge LP, Jonker CTH, Seifar RM, Heijnen JJ, van Gulik WM (2010) Intracellular metabolite determination in the presence of extracellular abundance: application to the penicillin biosynthesis pathway in *Penicillium chrysogenum*. Biotechnol Bioeng 107(1):105–115

Guo G, Tian P-P, Tang D, Wang X, Yang H-J, Cao P, et al. (2015) Metabolomics analysis between wild-type and industrial strains of *Streptomyces avermitilis* based on gas chromatography–mass spectrometry strategy. In Zhang T-C, Nakajima M, editors. Advances in applied biotechnology: proceedings of the 2nd international conference on applied biotechnology (ICAB 2014)-Volume II Berlin, Heidelberg: Springer Berlin Heidelberg, pp 477–485

Hong M, Mou H, Liu X, Huang M, Chu J (2017) ^{13}C-assisted metabolomics analysis reveals the positive correlation between specific erythromycin production rate and intracellular propionyl-CoA pool size in *Saccharopolyspora erythraea*. Bioprocess Biosyst Eng 40:1337–1348

Hwang K-S, Kim HU, Charusanti P, Palsson BØ, Lee SY (2014) Systems biology and biotechnology of *Streptomyces* species for the production of secondary metabolites. Biotechnol Adv 32(2):255–268

Japelt KB, Christensen JH, Villas-Boas SG (2015) Metabolic fingerprinting of *Lactobacillus paracasei*: the optimal quenching strategy. Microb Cell Fact 14:132–141

Kapoore RV, Coyle R, Staton CA, Brown NJ, Vaidyanathan S (2017) Influence of washing and quenching in profiling the metabolome of adherent mammalian cells: a case study with the metastatic breast cancer cell line MDA-MB-231. Analyst 142(11):2038–2049

Kassama Y, Xu Y, Dunn WB, Geukens N, Anne J, Goodacre R (2010) Assessment of adaptive focused acoustics versus manual vortex/freeze-thaw for intracellular metabolite extraction from *Streptomyces lividans* producing recombinant proteins using GC-MS and multi-block principal component analysis. Analyst 135(5):934–942

Koning WD, Dam KV (1992) A method for the determination of changes of glycolytic metabolites in yeast on a subsecond time scale using extraction at neutral pH. Anal Biochem 204(1):118–123

Lameiras F, Heijnen JJ, van Gulik WM (2015) Development of tools for quantitative intracellular metabolomics of *Aspergillus niger* chemostat cultures. Metabolomics 11:1253–1264

Link H, Anselment B, Weuster-Botz D (2008) Leakage of adenylates during cold methanol/glycerol quenching of *Escherichia coli*. Metabolomics 4(3):240–247

Lu H, Liu X, Huang M, Xia J, Chu J, Zhuang Y et al (2015) Integrated isotope-assisted metabolomics and ^{13}C metabolic flux analysis reveals metabolic flux redistribution for high glucoamylase production by *Aspergillus niger*. Microb Cell Fact 14(1):147–160

Lu C, Zhang X, Jiang M, Bai L (2016) Enhanced salinomycin production by adjusting the supply of polyketide extender units in *Streptomyces albus*. Metab Eng 35:129–137

Maharjan RP, Ferenci T (2003) Global metabolite analysis: the influence of extraction methodology on metabolome profiles of *Escherichia coli*. Anal Biochem 313(1):145–154

Mashego M, Van Gulik W, Vinke J, Heijnen J (2003) Critical evaluation of sampling techniques for residual glucose determination in carbon-limited chemostat culture of Saccharomyces cerevisiae. Biotechnol Bioeng 83(4):395–399

Min HS, Lee DY, Liu KH, Fiehn O, Kim KH, Chem A (2010) Evaluation of sampling and extraction methodologies for the global metabolic profiling of *Saccharophagus degradans*. Anal Chem 82(15):6660–6666

Muhamadali H, Xu Y, Ellis DI, Trivedi DK, Rattray NJW, Bernaerts K et al (2015) Metabolomics investigation of recombinant mTNFα production in *Streptomyces lividans*. Microb Cell Fact 14(1):157–168

Reichardt C (1979) Empirical parameters of solvent polarity. Angew Chem Int Ed 18(2):98–110

Seifar RM, Ras C, van Dam JC, van Gulik WM, Heijnen JJ, van Winden WA (2009) Simultaneous quantification of free nucleotides in complex biological samples using ion pair reversed phase liquid chromatography isotope dilution tandem mass spectrometry. Anal Biochem 388(2):213–219

Seifar RM, Ras C, Deshmukh AT, Bekers KM, Suarez-Mendez CA, da Cruz ALB et al (2013) Quantitative analysis of intracellular coenzymes in *Saccharomyces cerevisiae* using ion pair reversed phase ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr A 1311:115–120

Smart KF, Aggio RB, Van Houtte JR, Villas-Bôas SG (2010) Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry. Nat Protoc 5(10):1709–1729

Taymaz-Nikerel H, De MM, Ras C, Ten PA, Seifar RM, van Dam JC et al (2009) Development and application of a differential method for reliable metabolome analysis in *Escherichia coli*. Anal Biochem 386(1):9–19

van Dam JC, Eman MR, Frank J, Lange HC, van Dedem GW, Heijnen SJ (2002) Analysis of glycolytic intermediates in *Saccharomyces cerevisiae* using anion exchange chromatography and electrospray ionization with tandem mass spectrometric detection. Anal Chim Acta 460(2):209–218

Villasbôas SG (2005) Global metabolite analysis of yeast: evaluation of sample preparation methods. Yeast 22(14):1155–1169

Villas-Bôas SG, Bruheim P (2007) Cold glycerol–saline: the promising quenching solution for accurate intracellular metabolite analysis of microbial cells. Anal Biochem 370(1):87–97

Villas-Boas SG, Mas S, Akesson M, Smedsgaard J, Nielsen J (2005) Mass spectrometry in metabolome analysis. Mass Spectrom Rev 24(5):613–646

Wang B, Liu J, Liu H, Huang D, Wen J (2015) Comparative metabolic profiling reveals the key role of amino acids metabolism in the rapamycin overproduction by *Streptomyces hygroscopicus*. J Ind Microbiol Biotechnol 42(6):1–15

Wentzel A, Sletta H, Consortium S, Ellingsen TE, Bruheim P (2012) Intracellular metabolite pool changes in response to nutrient depletion induced metabolic switching in *Streptomyces coelicolor*. Metabolites 2(1):178–194

Winder CL, Dunn WB, Schuler S, Broadhurst D, Jarvis R, Stephens GM et al (2008) Global metabolic profiling of *Escherichia coli* cultures: an evaluation of methods for quenching and extraction of intracellular metabolites. Anal Chem 80(8):2939–2948

Wordofa GG, Kristensen M, Schrübbers L, Mccloskey D, Forster J, Schneider K (2017) Quantifying the metabolome of *Pseudomonas taiwanensis* VLB120: evaluation of hot and cold combined quenching/extraction approaches. Anal Chem 89:8738–8747

Wu L, Mashego MR, van Dam JC, Proell AM, Vinke JL, Ras C et al (2005) Quantitative analysis of the microbial metabolome by isotope dilution mass spectrometry using uniformly ^{13}C-labeled cell extracts as internal standards. Anal Biochem 336(2):164–171

Xia ML, Huang D, Li SS, Wen JP, Jia XQ, Chen YL (2013) Enhanced FK506 production in *Streptomyces tsukubaensis* by rational feeding strategies based on comparative metabolic profiling analysis. Biotechnol Bioeng 110(10):2717–2730

Zhou YJ, Li JL, Zhu J, Chen S, Bai LQ, Zhou XF et al (2008) Incomplete beta-ketone processing as a mechanism for polyene structural variation in the FR-008/candicidin complex. Chem Biol 15(6):629–638

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