Lysis and Extraction of Biomolecules from Eukaryotic and Prokaryotic Cells Powered by AFA-energetics®

Abstract

This technical note describes a novel automatable approach for the extraction of biomolecules from microbial samples for analysis with mass spectrometry technologies such as MALDI-TOF or LC-MS. Lysing cells and subsequent extraction of metabolites and proteins is achieved on a Covaris LE220-plus Focused-ultrasonicator using a non-contact method mediated by Adaptive Focused Acoustics® (AFA®). Rapid lysis and extraction occur in less than 2 minutes for 96 individual bacteria samples, and 12 minutes for 96 individual yeast samples when using a 96 AFA-TUBE TPX Plate. Mass spectra reveal that this is a highly sensitive and exceedingly reproducible method for the high throughput extraction of biomolecules from microbial samples.

Introduction

Proteomics, the study of proteins, and metabolomics, the study of small intermediate molecules and products of cell metabolism, are important techniques employed in a variety of industrial applications. In drug design and development, biomolecules are analyzed to determine the efficacy and toxicity of new medicines [1,2]. Altering the metabolism of model organisms like *E. coli* and *S. cerevisiae* allow for the engineering of organic factories that can be modified to produce anything from food and drugs to essential biofuels [3]. Metabolomics and proteomics are also utilized in food science, whether for the identification of pathogens and pesticides [4,5], or to brew specialty beers and wines [6].

The basic workflow of biomolecule analysis starts with the preparation of cells or tissues, followed by extraction of samples and analysis with nuclear magnetic resonance (NMR) or mass spectrometry (MS) [7]. MS functions by ionizing the molecules in a sample before separating them based on their mass and charge. The high concentrations of salts and detergents commonly found in lysis buffers are incompatible with mass spectrometry and must therefore be removed or diluted from the sample before performing the assay [8]. Here, we applied MS because the technique is more capable of detecting low abundance metabolites than NMR and allows for the identification of molecules based on their mass.

Biomolecule extraction can be very laborious, fallible, and time-consuming [9]. Standard methods generally utilize bead beating or tissue homogenizers for which rising sample temperatures are an issue [10]. Alternative techniques require the use of solvents like chloroform and methanol cooled to temperatures below -40 °C, and can demand up to an hour for the completion of a single extraction [11]. Covaris extraction methods are thermostable, highly reproducible, and capable of isolating metabolites and proteins from up to 96 different samples in as little as 2 minutes. ***Table 1*** presents a comparison of current extraction methods.

Company/ Method	Kit	Prep Time	Extraction Volume	Automation Available? (Y/N)	Sample
Covaris	truXTRAC	2 to 12 min per plate	30 to 40 µL	Y	1 x 10^6 bacteria or 1 x 10^7 yeast cells
Sigma [12]	Fatty Acid Extraction Kit	5 min per sample	3.5 mL	N	5 to 150 mg tissue
Bertin [13]	Precellys C14 Homogeniza-tion Kit	5 to 10 min for 1 to 24 samples	0.5 to 7 mL	N	1 mg to 2 g tissue / 5 µL to 6 mL culture
Cold methanol [11]	Manual	40 min +	5 mL +	N	5 mL culture +
Boiling Ethanol [11]	Manual	30 min +	5 mL +	N	5 mL culture +

Table 1. Comparison of Covaris biomolecule extraction protocol to current alternative methods and available kits.

In this technical note, we provide guidelines for highly reproducible sample preparation protocols using the Covaris LE220-plus/ LE220R-plus Focused-ultrasonicators and the 96 AFA-TUBE TPX plate. This new application allows for the rapid extraction of proteins and metabolites from a variety of microbes concurrent with cell lysis in a high throughput approach that is fully automatable. The AFA-energetics process allows for efficient, non-contact, and temperature controlled mechanical disruption of cells, which leads to a high yield/high-quality extraction. Samples extracted using Covaris AFA technology are compatible with analysis via mass spectrometry. ***Figure 1*** depicts the basic workflow for cell lysis.

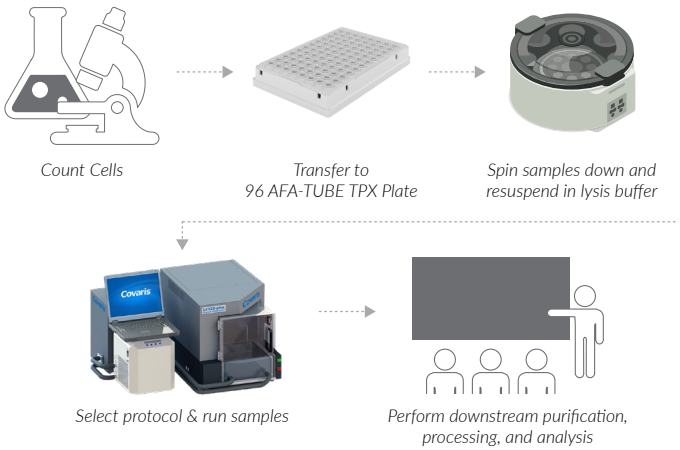

Figure 1. Workflow detailing sample preparation steps for extraction of proteins and metabolites from cells.

Materials and Methods

Required Materials

Covaris

- LE220-plus Focused-ultrasonicator (PN 500569)
- 96 AFA-TUBE TPX Plate (PN 520272)
- 96 microTUBE Plate Thin Foil Seals (PN 520235)
- PS Rack 96 AFA-TUBE TPX Plate (PN 500622)
- truXTRAC® Extraction Solvent (PN 190355)

Other

- Acetonitrile (Thermo Fisher Scientific, PN 85176)
- Trifluoroacetic acid (Thermo Fisher Scientific, PN 28904)
- ZipTip with C18 Resin (EMD Millipore, PN ZTC18S096)
- MALDI-TOF Mass Spectrometer (Bruker, microflex LT)
- MALDI Target Plate (Bruker, PN 8280800)
- α-Cyano-4-hydroxycinnamic acid (Sigma-Aldrich, PN C2020-10G)
- Refrigerated centrifuge (Eppendorf, PN 5810 R)

Methods

Covaris truXTRAC Extraction Solvent was used to resuspend cells in a 96 AFA-TUBE TPX Plate. The cells were subsequently lysed on a LE220-plus Focused-ultrasonicator according to the settings described in ***Table 2***. The cell lysate was desalted with ZipTip pipette tips (EMD Millipore) according to the manufacturer's protocol and eluted in 70% acetonitrile / 0.1% TFA. Eluate was mixed 1:1 with a saturated solution of α-Cyano-4-hydroxycinnamic acid in 50% acetonitrile / 1% TFA, before pipetting 1 µL sample onto the target plate. MALDI analysis was performed in positive ion linear mode with a nitrogen laser (337 nm) set to 30% power, firing 1000 shots per sample in a randomized pattern. The number of cells per well of the 96 AFA-TUBE TPX Plate ranged from 1 x 10^7 cells for yeast to 1 x 10^8 cells for bacteria.

Instrument	LE220-plus Focused-ultrasonicator
Vessel	96 AFA-TUBE TPX Plate
Rack	PS Rack 96 AFA-TUBE TPX Plate (PN 500622)
Dithering	1.0 mm y-dither @ 20 mm/s
Temperature	10 °C
Peak Incident Power	450W
Duty Factor	50%
Cycles per Burst	200
Treatment Time	2s AFA, 1 s delay repeated for 1 min per row

Table 2. AFA treatment settings for cell lysis.

Results

Bacteria Lysis

The mass spectra obtained following the lysis of a single *E. coli* colony grown overnight on LB agar presents sharp, distinct peaks from 2 to 20 kDa as seen in ***Figure 2***. A manual analysis of this spectrum was performed utilizing the *E. coli* Metabolome Database [14] and UniProt [15] to tentatively identify a number of peaks as proteins and metabolites essential to cell growth including a 50S ribosomal protein (12.3 kDa), a subunit of DNA polymerase (8.8 kDa), and two components of surface membrane lipopolysaccharides (3.6 kDa and 3 kDa). ***Figure 3*** compares the mass spectrum obtained following lysis of 1 x 10^8 *E. coli* cells grown to exponential phase in LB media to the spectrum obtained from *E. coli* grown in M9 minimal media. The aforementioned signals are present in both samples, but a number of peaks between 1 and 5 kDa appear only for cells grown in LB. This disparity between samples is expected because bacteria are known to produce different biomolecules when grown under various environmental stressors such as extreme pH or a limited carbon source, as well as when forced to grow slowly [16].

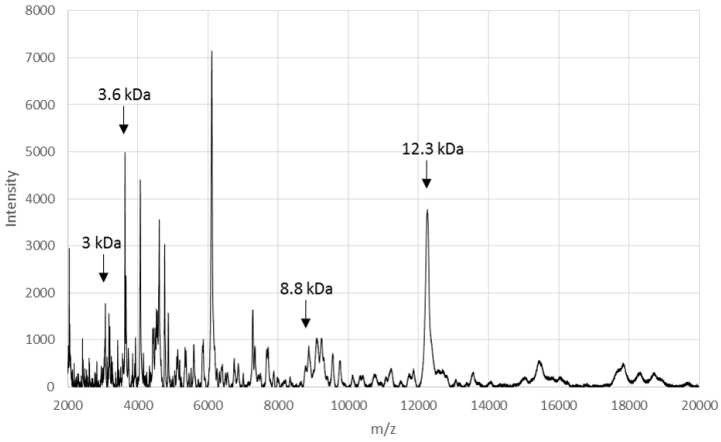

Figure 2. Mass spectrum of metabolites and proteins extracted from a single *E. coli* colony grown overnight on LB agar.

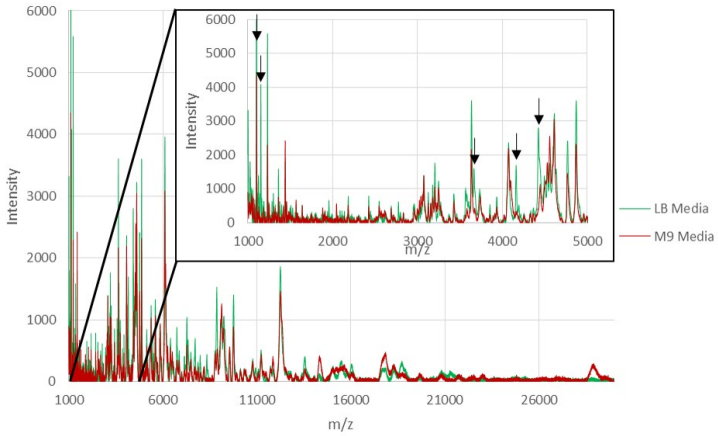

Figure 3. Superposition of mass spectra from metabolites and proteins extracted from *E. coli* grown overnight in LB media (Green) and in M9 minimal media (Red). Arrows indicate peaks below 5 kDa which appear only in spectra of cells grown in LB.

Yeast Lysis

The mass spectrum obtained following lysis of 1 x 10^7 *S. cerevisiae* cells grown to exponential phase in YM media is compared to the spectrum obtained from *S. cerevisiae* grown to stationary phase in ***Figure 4***. A manual analysis of these spectra utilizing the Yeast Metabolome Database [17] and Saccharomyces Genome Database [18] tentatively identified a number of peaks as proteins and metabolites essential to cell growth including two ribosomal subunits (60S, 13.9 kDa; and 40S, 15.8 kDa), cardiolipin (1.4 kDa), and N-acetyl-D-glucosaminyldiphosphodolichol (1.7 kDa). The disparity between these spectra, for example loss of the 40S ribosomal subunit upon transition to stationary phase, is indicative of a shift in metabolism necessary to accommodate a nutrient-deficient environment. For instance, when yeast cells are no longer growing rapidly, the synthesis and degradation of ribosomes are regulated such that the number of ribosomes per cells declines to less than 25% of the standard level [19].

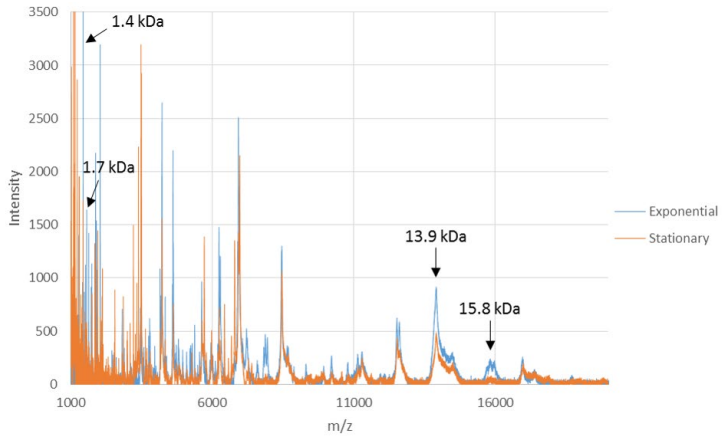

Figure 4. Superposition of mass spectra from metabolites and proteins extracted from *S. cerevisiae* in the exponential (Blue) and stationary (Orange) growth phase.

Conclusions

We have shown reproducible and robust data for the extraction of proteins and metabolites from a variety of cell types using optimized protocols as outlined in the Materials and Methods. Cultured cells present little variability from day to day and demonstrate nearly identical mass spectra as compared to fresh colonies selected from agar plates. The automatability of this application presents a significant advantage for large-scale metabolomics studies which require analysis of many thousands of samples. Drug discovery and design, and investigations into the metabolic response of cells to large chemical and drug libraries are becoming more common, necessitating the development of reliable high throughput extraction technologies [20].

The protocols presented above utilize the 96 AFA-TUBE TPX Plate on the Covaris LE220-plus Focused-ultrasonicator to quickly perform cell lysis and extraction with no transfer steps until analysis or final storage of the product. The method is robust and flexible, capable of extracting sufficient sample from bacteria with a single 2-second pulse or from yeast in 1 minute. Researchers interested in high throughput metabolomics can apply the protocols outlined in this technical note to improve the speed and consistency of their research.

References

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