BIOTRANS
LA ROCHELLE 2023

N°1611 / PC
TOPIC(s) : Enzyme production, immobilization

In search of complementary extraction methods for comprehensive coverage of metabolites in Escherichia coli

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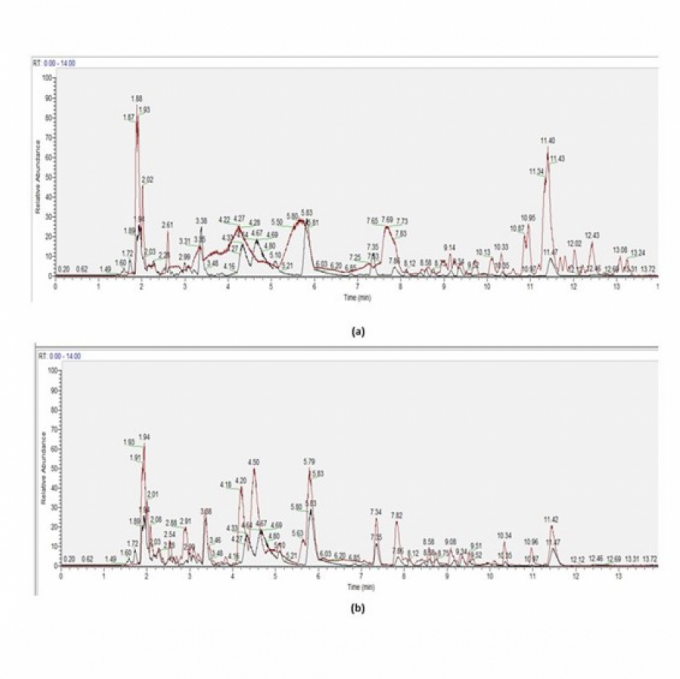
PURPOSE OF THE ABSTRACT

Escherichia coli is an invaluable research tool for many fields of biology, in particular for its use in expressing recombinant proteins for industrial processes [1]. However, the activity of many such enzymes cannot be determined using standard biochemical assays, as often the relevant substrates are not known, or the products produced are not detectable. Today, the biochemical footprints of such unknown enzyme activities can be revealed via analysis of the metabolomes of the recombinant E. coli clones employed, using sensitive technologies such as mass spectrometry [2]. Before any valuable metabolites can be identified and produced at large scale, it is necessary to achieve as high a coverage of the potential metabolites present within E. coli as possible. Current methods for metabolomics usually involve 1 extraction method and attempting to maximise its efficiency of metabolite recovery [3], [4, 5]. Very commonly these methods also involve a drying step to concentrate the metabolites for better detection, but this removes a large subset of metabolites (see Figure 1). We have therefore analysed a wide range of different extraction methods against the cell free extracts of various recombinant E. coli clones. The results were analysed to determine the minimum number of extractions that achieved high recovery and coverage of metabolites, thus maximising the chance of pinpointing novel metabolites. This revealed that two extraction methods produce significant differences in the metabolomes of the 5 recombinant E. coli clones analysed. These two methods were chosen due to their ability to produce not only high numbers of ions, but wide mass coverage and a high degree of complementarity (Figure 2). One extraction method uses methanol and water, in a 4:1 ratio, which was then dried down and reconstituted in the chromatography running buffer and the other extraction method uses a combination of methanol, water, and chloroform, in a 3:1:1 ratio, that was injected directly onto the chromatography column.

FIGURES



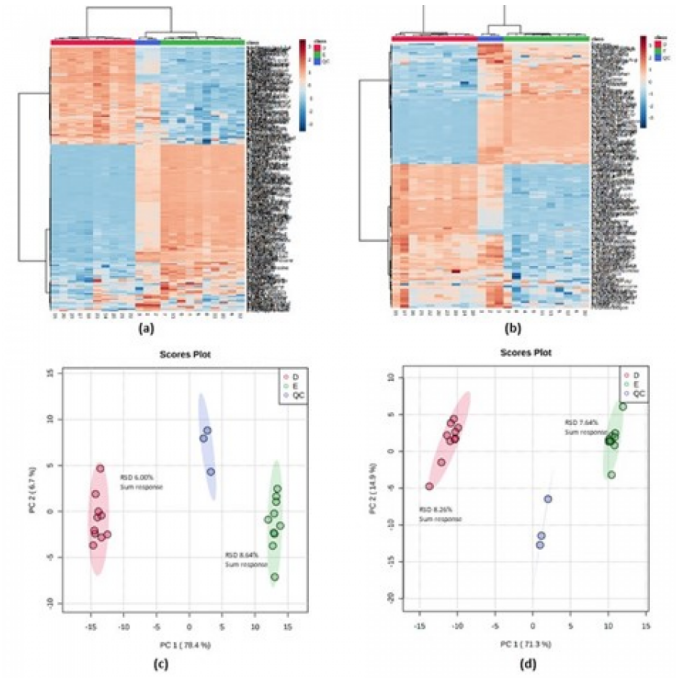


FIGURE 1

Figure 1

(a) Chromatogram for sample extracted using Methanol:Chloroform:Water extraction solvent (no drying step) (red) and Methanol:Water extraction solvent (with a drying step and reconstitution) (Black). (b) Chromatogram for sample extracted using Methanol:Chl

FIGURE 2

Figure 2

Heat maps showing metabolite abundance for (a) positive mode and (b) negative mode chromatography. For positive mode, 211 features were identified out of 2580 MS2 features < 25% RSD, for negative mode 151 features were identified out of 1864 MS2 features

KEYWORDS

Enzyme production | Metabolites

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