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MetaNetter 2: A Cytoscape plugin for ab initio network analysis and metabolite feature classification

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ABSTRACT

Metabolomics frequently relies on the use of high resolution mass spectrometry data. Classification and filtering of this data remain a challenging task due to the plethora of complex mass spectral artefacts, chemical noise, adducts and fragmentation that occur during ionisation and analysis. Additionally, the relationships between detected compounds can provide a wealth of information about the nature of the samples and the biochemistry that gave rise to them.

We present a biochemical networking tool: MetaNetter 2 that is based on the original MetaNetter, a Cytoscape plugin that creates ab initio networks. The new version supports two major improvements: the generation of adduct networks and the creation of tables that map adduct or transformation patterns across multiple samples, providing a readout of compound relationships.

We have applied this tool to the analysis of adduct patterns in the same sample separated under two different chromatographies, allowing inferences to be made about the effect of different buffer conditions on adduct detection, and the application of the chemical transformation analysis to both a single fragmentation analysis and an all-ions fragmentation dataset.

Finally, we present an analysis of a dataset derived from anaerobic and aerobic growth of the organism *Staphylococcus aureus* demonstrating the utility of the tool for biological analysis.

1. Introduction

Metabolomics is rapidly becoming a standard tool for 'omics' research. The application of high-resolution mass spectrometry systems such as the Orbitrap [1] and high resolution Q-ToFs [2] allow the generation of rich datasets with accurate mass information that allows substantial inference on the metabolite composition of a mixture to be obtained.

In complex mixtures, features detected using high resolution MS are often interpreted independently. These features are nevertheless related to each other since they may be involved in the same biochemical reactions (one being the substrate and the other the product). They also may be technologically connected due to ionisation modes (e.g. fragments, adducts). When annotating, it is important to take into account this information since it can provide valuable clues on the molecular nature of features. A common way to elucidate adducts and fragments consists in clustering peaks related to the same original compound [3]. By taking biochemistry into account, it is possible to exploit the constraints implied by the metabolic network (the union of all biochemical reactions which can occur in a metabolic network) [4]. To develop this network topology it is first necessary to establish a correspondence (mapping) between features and metabolites in the network. The overlap between features and metabolites in the network, however is far from satisfying, meaning that not all the features can be mapped in these networks. One of the reasons is that metabolic networks are inferred from genomic information and common knowledge on metabolism which can be incomplete. The other reason is that some metabolites like lipids are represented in the network by class compounds (e.g. "a sphingolipid").

To analyse all features in a network perspective, Breitling et al. [5] proposed to build "ab initio" networks based on mass differences. For each pair of features in the network, if the mass difference between them is equal (up to few ppm) to the mass difference of a biochemical reaction, then these two features can be connected. Following this work, we proposed software (a Cytoscape plugin) called MetaNetter [6] allowing this network reconstruction to be performed.

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The MetaNetter plugin for Cytoscape 2 provided the capability to perform ab initio network prediction and was in the top 50 downloaded apps. Cytoscape is a powerful software package for displaying and manipulating networks. The original MetaNetter relied on a configurable list of potential chemical transforms. When provided with a list of accurate masses and a mass tolerance to take the inherent noise affecting mass information into account, MetaNetter produces a graph specifying the individual masses as nodes in the network, where predicted transformations are depicted as edges as described in the original paper. This first release of the plugin is not compatible with the new version of Cytoscape (3.0). Moreover it only took into account the biochemical relationships between features and not adducts. This last functionality is important for metabolite annotation as will be shown in this article.

The new version has been substantially rewritten to adhere to the model provided by the latest version of Cytoscape (3.0). An adduct matching tool has been provided alongside the transformations method, allowing annotation of links between nodes that potentially derive from non-proton adducts. Additionally, retention time restriction has been added, allowing matches (predominantly for adduct matching) to be only allowed within a user-definable time window.

The original version of MetaNetter has also been successfully used to map features across multiple samples, allowing the chemical transformations in different states and under different experimental conditions to be observed, for example analysing the chemical relationships within dissolved organic matter from samples at the ocean surface compared to the deep ocean [7]. This new version also computes frequencies of each mass difference and adducts at a given threshold, generating a table which can highlight the overrepresentation of particular transformations, allowing this type of analysis to be performed rapidly and easily.

2. Methods and materials

2.1. Software

The MetaNetter 2 plugin was written in Java 1.8 using the MAVEN framework for compilation. MetaNetter 2 is an OSGI module compliant with the Cytoscape 3.0 application. MetaNetter 2 is available in the Cytoscape App Store.

2.2. Samples

5 ul of foetal calf serum was extracted using 200 uL ice cold chloroform/methanol/water 1:3:1. The resulting mixture was vortexed for 30 s followed by centrifugation for 5 min at 13,000*g*.

Standards mixes were prepared as described as in [8].

A clinical strain of *S. aureus*, 5817-q14, LHSKBClinical [9] was cultured overnight on Brain Heart Infusion (BHI) agar plates (Oxoid Limited, Basingstoke, UK) in a humidified static incubator at 37 °C. For the preparation of sub-cultures, single colonies were taken and inoculated into BHI broth media (Oxoid) in 1,5 mL reaction tubes (Eppendorff). Aliquots (1 mL) of these liquid cultures were then incubated overnight at 37 °C in a shaking incubator with 180 rpm to ensure a stationary growth phase.These were used for the subsequent preparation of biofilm and planktonic cell cultures. The planktonic cell cultures were grown in a shaking incubator with 180 rpm and 37 °C under aerobic and anaerobic conditions, the biofilm samples on the other hand were grown under aerobic conditions in a static incubator at 37 °C.

Planktonic cell and biofilm cell extracts were obtained following the planktonic and biofilm extraction protocols described in Ref. [10].

2.3. LC–MS methodology

10 uL of each cell extract was injected onto an UltiMate 3000 RSLC system (Thermo, UK) and separated using a 4 mm × 150 mm ZIC-pHILIC column (for high pH adduct analysis and fragmentation analysis) or a 4 mm × 150 mm ZIC-HILIC column (for low pH adduct analysis). All gradients and solvents were identical (A: water, B: acetonitrile) save for 20 mM ammonium carbonate, used as an ion pair in buffer A for the pHILIC analysis, and 0.1% formic acid added to buffer A and 0.08% formic acid added to buffer B in the HILIC analysis. Gradients started from 20% A, rising to 80% after 15 min with a step to 95% A for 3 min, followed by 6 min equilibration time.

3. Results

Similar to MetaNetter, MetaNetter 2 requires an input file in text format with a list of masses. For retention time matching a column containing these times for each peak must be supplied. For correlation sorting or visual mapping of the nodes by abundance, additional columns may be provided headed with a sample label and containing numerical values for abundances.

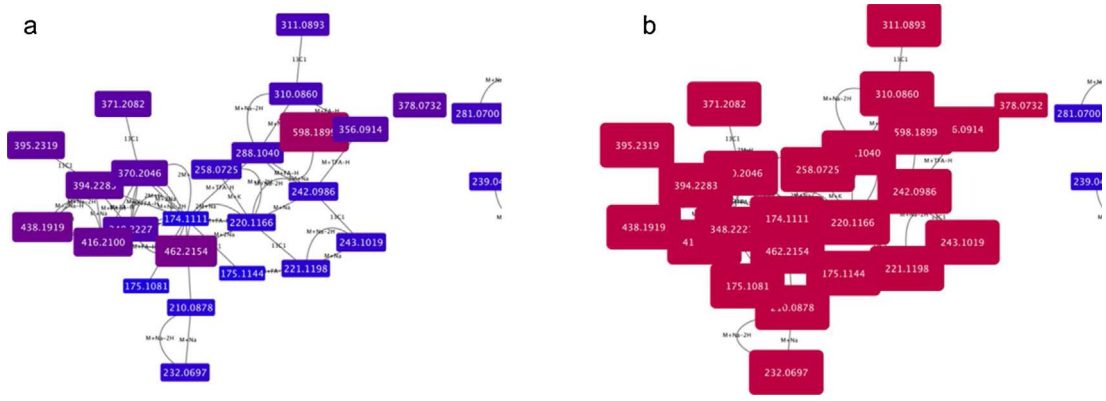

Fig. 1. a and b show the adduct pattern for a peak at 174.1111 (centre), corresponding to arginine. Fig. 1a is visually formatted by mass, with red coloured and larger nodes denoting greater masses than blue small nodes. Fig. 1b is visually mapped by retention time, demonstrating that all peaks mapped elute at the same time, towards the end of the run (denoted by the purple colour of the nodes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).