Chemometric evaluation of Saccharomyces cerevisiae metabolic profiles using LC–MS

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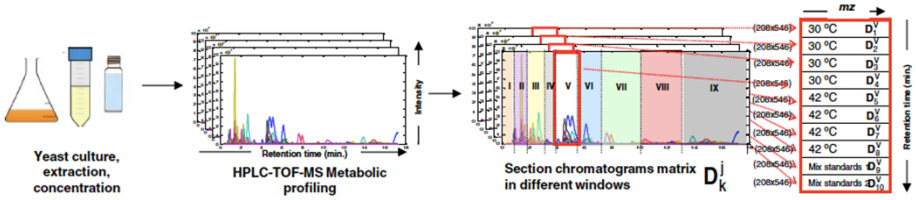
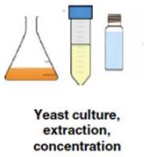
Outline of paper



What did they do:
1: Exposed yeast to 30/45°C for 1 hr
2: Extracted sample and ran through an LTQ Orbitrap with ESI injection (positive mode)
3: Analyzed samples



Yeast culture, extraction, concentration

Yeast strain W303a
+
blank sample

30/45°C for 1 hr

N=4

Cooled on ice, centrifuge, washed pellet with cold PBS

Added 2 standards to each tube

Centrifuge, washed pellet with cold PBS, remove supernatant

Add 75% EtOH, 3min at 80°C

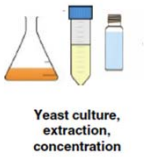
Evaporated, using N_2

Resuspended in .5mL (95% Acetonitrile), .2um filter

TSK gel Amide 80
(NH_2CO-Silica)

LC and Orbitrap

Solvent A: 0.5 mM ammonium acetate in 90 % acetonitrile at pH 5.5
Solvent B: 2.5 mM ammonium acetate in 60 % acetonitrile at pH 5.5



Yeast culture, extraction, concentration

Resuspended in .5mL (95% Acetonitrile), .2um filter

5uL

TSK gel Amide 80
(NH_2CO-Silica)

ESI
LTQ Orbitrap

50-1000da

Data collection

All data between 50-1000 daltons
Full data scan consisted of 3,587 retention times with 951 m/z data points (for each sample)

↓

Size reduced to include only m/z points between 55 and 600 Da

↓

Chromatograms interpolated to same retention time, and reduced to 2020 retention times (0-17mins)

↓

Baseline and background correction using mean chromatogram of blank sample. Analyzed by Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

Total ion current (TIC): sum of m/z per unit of time

Peak alignment using Correlation Optimization Warping (COW)

"the segment m, which is the length of the sections in which the chromatogram is divided, the slack size t, which is the maximum chromatographic peak warping allowed and a reference chromatogram."

TIC matrix data was mean centered after COW alignment

Multivariate curve resolution alternating least squares (MCR-ALS)

$$\mathbf{D}_k^j = \mathbf{C}_k^j \mathbf{S}^{j,T} + \mathbf{E}_k^j \text{ for } j = \text{I, II, ..., IX windows and } k = 1, 2, \ldots, 10 \text{ samples}$$

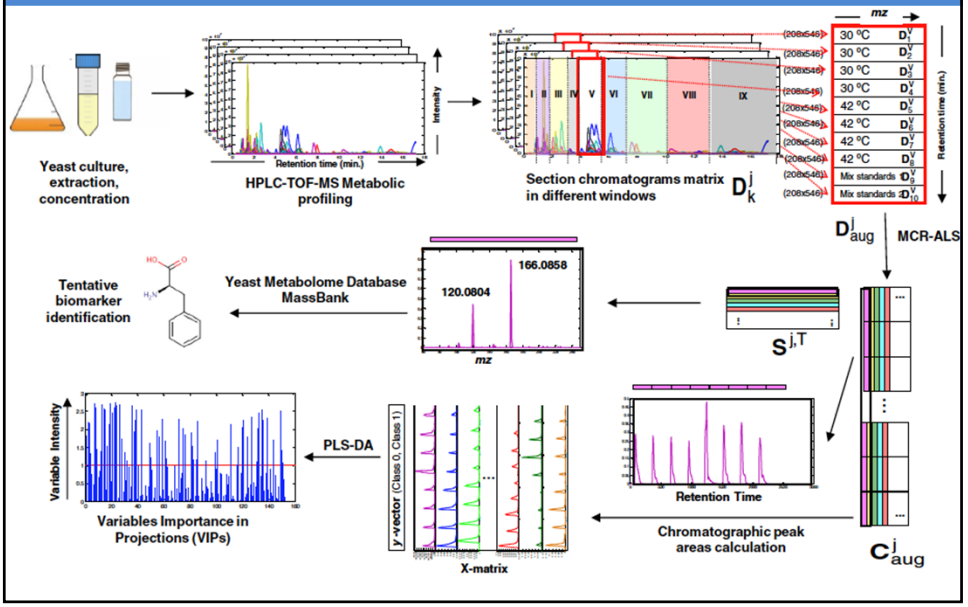
Rows of data matrices D(j/k) are the different elution times of the samples chromatographic analysis. Columns of data matrices D(j/k) are the mass spectra recorded at the different elution times. C(j/k) is the matrix of MCR-ALS resolved elution profiles in window j and sample k , and S(j/T) is the matrix of their corresponding pure mass spectra. These resolved pure mass spectra can be then used for the identification of the different metabolites. E(j/k) contains the unexplained variance related to background and noise contributions not modelled by C(j/k) and S(j/T) .

Multivariate curve resolution alternating least squares (MCR-ALS)

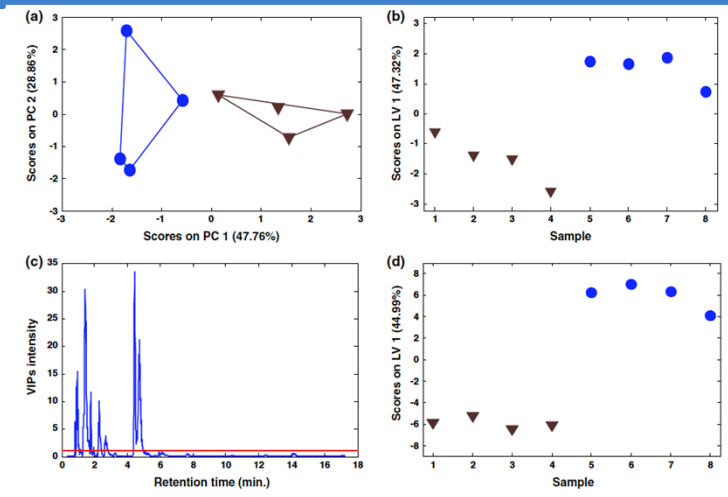
$$\mathbf{D}_{aug}^j = \begin{bmatrix} \mathbf{D}_1^j \\ \mathbf{D}_2^j \\ \mathbf{D}_3^j \\ \vdots \\ \mathbf{D}_{10}^j \end{bmatrix} = \begin{bmatrix} \mathbf{C}_1^j \\ \mathbf{C}_2^j \\ \mathbf{C}_3^j \\ \vdots \\ \mathbf{C}_{10}^j \end{bmatrix} \mathbf{S}^{j,T} + \begin{bmatrix} \mathbf{E}_1^j \\ \mathbf{E}_2^j \\ \mathbf{E}_3^j \\ \vdots \\ \mathbf{E}_{10}^j \end{bmatrix}$$

$$= \mathbf{C}_{aug}^j \mathbf{S}^{j,T} + \mathbf{E}_{aug}^j \text{ for } j = \text{I, II, ..., IX windows}$$

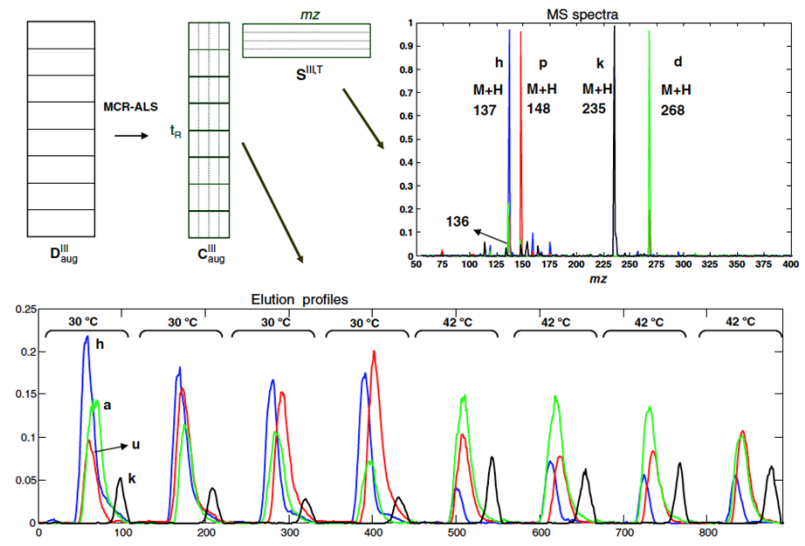
Workflow with MCR-ALS



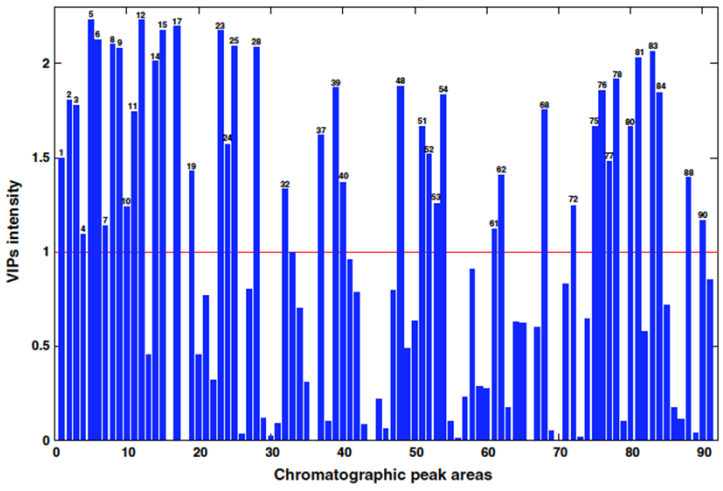
PLS-DA analysis using MS-TIC (a-c) and MCR-ALS (d)



Example of MCR-ALS plot



Variable importance in projection score from PLS-DA vis MCR-ALS alignment



List of metabolites identified

Peak number	C-number	Metabolite	Weight	Peak number	C-number	Metabolite	Weight
3	C06104	Adipic acid	0.1401	1	C00033	Acetic acid	−0.1285
5	C05853	2-Phenylethanol	0.1568	2	C00097	L-Cysteine	−0.141
6	C00077	L-Ornithine	0.1532	4			−0.1098
11	C00864	Pantothenate	0.1388	7	C00116	Glycerol	−0.1121
12	C01571	Capric acid	0.1569	8	C00147	Adenine	−0.1523
14	C00474	Arabitol/ribitol	0.1491	9	C00262	Hypoxanthine	−0.1516
15	C00559	Deoxyadenosine	0.1549	10	C00249	Palmitic acid	−0.1169
17	C06423	Caprylic acid	0.1558	23	C00791	Creatinine	−0.155
19	C00120	Biotin	0.1255	24			−0.1315
25	C00474	Arabitol/ribitol	0.1518	32		LysoPC(18:1(11Z))	−0.1213
28	C01087	2-Hydroxyglutaric acid	0.1517	37	C00902	2-Oxohexanoic acid	−0.1337
40	C00079	L-Phenylalanine	0.1229	39	C00160	Glycolic acid	−0.1437
51			0.1356	48	C00250	Pyridoxal	−0.1439
54	C00049	L-Aspartic acid	0.1423	52	C02794	L-3-Hydroxykynurenine	−0.1293
62	C00041	L-Alanine	0.1247	53	C00082	L-Tyrosine	−0.1176
72			0.1173	61	C00152	L-Asparagine	−0.1112
75	C00114	Choline	0.1356	68			−0.1392
76	C07113	Acetophenone	0.1432	77	C02059	Phylloquinone	−0.1277