Investigating Phytophthora methylation using Trapped Ion Mobility

ASMS 2019, Poster WP103

Authors

Han Chen[1]; Qing Zhang[2]; Heiner Koch[3]; Lucy Woods[3] Hongyu Ma[1]

[1]Nanjing Agriculture University, Nanjing, Jiangsu, China
[2]Bruker Daltonics, Shanghai, China
[3]Bruker Daltonics, Bremen, Germany

Introduction

Histones and their variants play import roles in cell metabolism in the point view of epigenetics. Post-Translational-Modification (PTM) is a critical factor for the biological function of histones. Investigating different kind of PTMs is critical and still an ongoing challenge in epigenetics due to the complexity of PTMs in histones. Moreover, it is general accepted that not only the type of PTM but also the relative ratio of PTMs could play a critical role in cell metabolism.

Mass spectrometry is now a keystone of investigation of histone PTMs due to its sensitivity and high-throughput. Scan speed is critical for maximizing identification and quantification information. Here we show how a QTOF equipped with trapped ion mobility using the PASEF acquisition mode operating at 120Hz in MSMS mode produced high sequence coverage and in-depth, quantitative PTM information on histone proteins from Phytophthora sojae.

Experimental

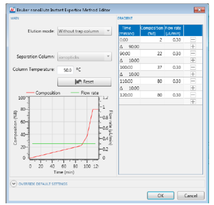
Sample preparation

Histone proteins from two samples (Ps and Pi) were extracted from *Phytophthora sojae* (Nanjing Agriculture University) using EpiOuik Total Histone Extraction Kit (Epigentek, NewYork, United States),. Extracted proteins were separated in 12% SDS-PAGE and dyed with Coomassie blue. Gel bands near 17K were cut and then digested by trypsin. Digested peptides were loaded into a 75um*25cm column (Ionoptics, Australia) on a nanoUHPLC system (nanoElute, Bruker Daltonics) coupled with a trapped ion mobility equipped QTOF (timsTOF Pro, Bruker Daltonics). Data were acquired in PASEF mode with MSMS scan speeds up to 120 Hz. Data were searched in PeaksX (Bioinformatics Solutions Inc.) against a customized database containing 6 histone variants.

MS scan mode

Bruker timsTOF Pro mass spectrometer
Acquisition mode: standard PASEF mode
Mass range: m/z 100-1700

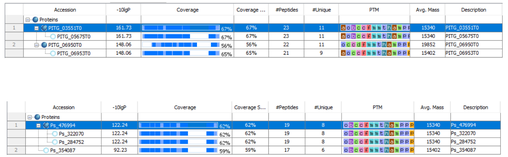
LC gradient



Results and Discussion

Four out of six proteins were identified by MSMS database search with sequence coverage above 60% (Fig. 1). For sample Ps, 66 peptides and 31 unique peptides were identified, For sample Pi, it was 36 peptides and 14 unique peptides.

Fig.1 Sequence coverage of measured samples.



Accession	-10lgP	Coverage	Coverage ...	#Peptides	#Unique	PTM	Avg. Mass	Description
Proteins								
PITG_03551T0	161.72		67%	23	11		15340	PITG_03551T0
PITG_05675T0	161.73		67%	23	11		15340	PITG_05675T0
PITG_06950T0	148.06		96%	22	11		19852	PITG_06950T0
PITG_06953T0	148.06		65%	21	9		15402	PITG_06953T0

Accession	-10lgP	Coverage	Coverage S...	#Peptides	#Unique	PTM	Avg. Mass	Description
Proteins								
Ps_476994	122.24		62%	19	8		15340	Ps_476994
Ps_320370	122.24		62%	19	8		15340	Ps_320370
Ps_284752	122.24		62%	19	8		15340	Ps_284752
Ps_354087	92.23		59%	17	6		15402	Ps_354087

Since for its high MSMS scan speed (>120 Hz), timsTOF Pro could get more PSM number which is critical for PTM site analysis. In this analysis, 399 and 238 PSMs were assigned to 18 kinds of PTM including critical modifications such as methylation, di-methylation and tri-methylation. Other rarely modification such as butyryl, crotonylation were also overserved (Fig. 2).

Fig.2 PTM sites of measured samples.

	ΔM	PTM	#
F	+27.99	Formylation	70
Me	+14.02	Methylation(KR)	70
Me	+14.02	Methylation(others)	58
P	+71.04	Propionamide (K, X@N-term)	42
Ci	+0.98	Citrullination	28
Ac	+42.01	Acetylation (K)	27
di	+28.03	Dimethylation(KR)	23
P	+79.97	Phosphorylation (STY)	19
Me	+14.02	Methylation(Protein N-term)	15
O	+15.99	Oxidation (M)	14
2h	+86.04	2-Hydroxyisobutyrylation	11
Cr	+68.03	Crotonylation	6
tri	+42.05	Trimethylation	5
Bu	+70.04	Butyryl	4
P	+79.97	Phosphorylation (HCDR)	3
Ma	+86.00	Malonylaiton-K	2
Ac	+42.01	Acetylation (Protein N-term)	1
U	383.23	Ubiquitination	1

	ΔM	PTM	#
F	+27.99	Formylation	46
Me	+14.02	Methylation(others)	38
Me	+14.02	Methylation(KR)	30
di	+28.03	Dimethylation(KR)	20
P	+79.97	Phosphorylation (STY)	19
P	+71.04	Propionamide (K, X@N-term)	19
Ac	+42.01	Acetylation (K)	12
Ci	+0.98	Citrullination	11
Me	+14.02	Methylation(Protein N-term)	11
O	+15.99	Oxidation (M)	10
Ma	+86.00	Malonylaiton-K	5
Cr	+68.03	Crotonylation	4
2h	+86.04	2-Hydroxyisobutyrylation	4
tri	+42.05	Trimethylation	3
P	+79.97	Phosphorylation (HCDR)	3
Bu	+70.04	Butyryl	2
U	383.23	Ubiquitination	1

For single protein such as PITG03551T0(Fig. 3) the sequence coverage is 67% and 15 kinds of PTMs was found. PTM on lysine is a important modification for biological function in plant, in this experiment, most of PTM were fond on lysine, show great potential of timsTOF Pro on PTM profiling. Based on number of PSMs we could get quantification information of PTM(Fig. 4), in protein PITG03551T0, The PTM ratios were determined as 72% methylation, 55% dimethylation and 8% trimethylation compared with unmodified if considered separately. When compared across different kinds of methylation, mono-methylation was about 56%, di-methylation 14% and tri-methylation 2.5 %, and unmodified 26%. Different kinds of methylation and relative ratios could help to explain some key metabolic functions. And for the other protein of Ps, the ratio was76% and 4.2% for mono and di-methylation but no tri-methylation were identified. This may indicate some specific biological function

Fig.3 Sequence coverage and PTM sites of PITG03551T0.

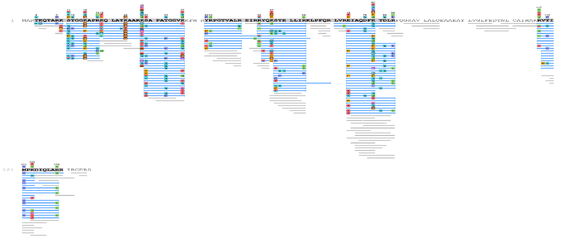
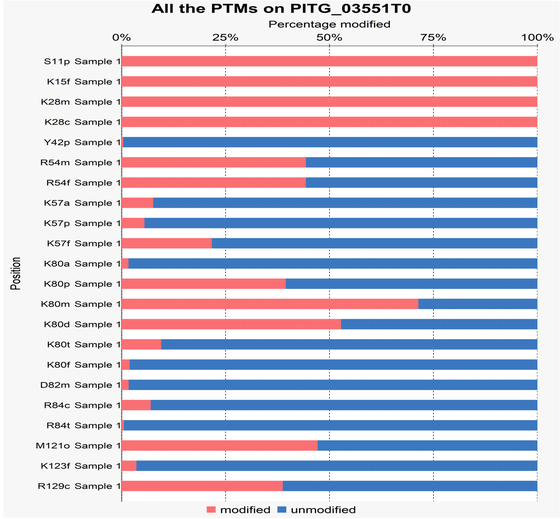


Fig.4 PTM profiling of PITG_03551T0



- A simple and reliable method for determination PTM sites and its ratio has been developed.
- The ultra-fast scan speed of timsTOF Pro combined with PASEF unveils a yet unseen depth of PTMs on extracted histone proteins.

timsTOF Pro