Geobacillus stearothermophilus CULTURE TECHNIQUE

Phospholipid analyses by MALDI-TOF/TOF mass spectrometry using 1,5-diaminonaphthalene as matrix

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Abstract

Geobacillus stearothermophilus **was grown at 60 °C, 300 rpm in a 5 L Winpact bench-top fermentor. With 1,5-diaminonaphthalene (DAN) as MALDI matrix, phospholipid spectra were free of matrix interference, with ~5–10× higher S/N at lower laser energy than DHB or 9AA; PE and PG were the major classes.**



Winpact Model: 5 L bench-top fermentor

Introduction

Motivation: phospholipids (PLs) make up 50–60% of membrane lipid mass and regulate cell signaling, growth and death; MALDI-MS offers high sensitivity, salt tolerance and throughput for lipidomics of complex biological samples.

Gap: chemical background noise in the low-mass region limits MALDI lipid analysis; the common matrices DHB and 9AA produce matrix or alkali adduct ions, and 9AA works only in negative ion mode.

Objective: evaluate 1,5-diaminonaphthalene (DAN) against DHB and 9AA on six PL standards in both ion modes, then apply it to PL extracts from fermentor-grown *G. stearothermophilus*.

Materials and Methods

Biomass production:

G. stearothermophilus NCTC 10003 was cultured in a 5 L Winpact bench-top fermentor (Major Science, USA) at 60 °C and 300 rpm. Cells were harvested at mid-exponential phase (OD600 ≈ 0.6) by centrifugation at 6000 × g for 10 min and the pellet was stored at −20 °C.

Lipid extraction:

Modified Bligh–Dyer method: 0.1 g cells in 1.2 mL $CHCl_3$/MeOH (2:1, v/v), sonicated 10 min, phase-separated with 0.4 mL water and centrifuged at 8000 × g for 10 min; the aqueous phase was re-extracted twice with 0.8 mL $CHCl_3$. Pooled organic phases were dried under nitrogen, redissolved in 1.0 mL $CHCl_3$/MeOH (2:1) and stored in the dark at −80 °C; extraction was kept at 4 °C to limit oxidation and hydrolysis.

MALDI-TOF/TOF analysis:

DAN (5 mg/mL in isopropanol/acetonitrile 60:40), DHB (30 mg/mL in methanol) and 9AA (10 mg/mL in isopropanol/acetonitrile) were premixed 1:1 with samples. Spectra were acquired on an AB Sciex 4800 MALDI-TOF/TOF (355 nm Nd:YAG, 200 Hz, 20 kV, reflector mode, m/z 400–1200) in positive and negative ion modes, using DMPC, DPPE, DPPI, DMPS, DMPA and DPPG standards; species were assigned by MS/MS with the LIPID MAPS database.

Results

DAN's own ions appeared below m/z ~460, leaving the PL range (m/z >650) free of matrix signals. DMPC in positive mode gave an almost exclusive $[M+H]^+$ with DAN, whereas DHB produced sodium adducts above 20% and 9AA needed much higher laser energy (>6000 vs ~4000 for DAN). DPPE in negative mode gave a single clean $[M-H]^-$ ion at m/z 662.5. In an equimolar mixture of six PLs, DAN increased mean S/N about 5–10-fold over DHB and 9AA in negative mode. Crude extracts spotted without pre-separation showed PE (m/z 648–704) and PG (m/z 649–749) as the major classes, with no PC detected. Fermentor relevance: controlled thermophilic cultivation at 60 °C and 300 rpm, harvested at a defined mid-exponential OD, gave consistent cell material for membrane lipid profiling; the high growth temperature also lowers contamination risk and supports high cell density.

References

Wei Dong, Qing Shen, Joewel T. Baibado, Yimin Liang, Ping Wang, Yeqing Huang, Zhifeng Zhang, Yixuan Wang, Hon-Yeung Cheung. Phospholipid analyses by MALDI-TOF/TOF mass spectrometry using 1,5-diaminonaphthalene as matrix. International Journal of Mass Spectrometry (2013) 343–344:15–22

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