
Generating new MSP with Bruker Microflex LT

Goal

To generate and validate new MSPs in order to complement the commercial Bruker database for microbial identification.

Equipment

MALDI microflex LT
Table top centrifuge (14000x g)
Vortex
Fume hood

Materials

Eppendorf tubes
Inoculation loops
Pipettes and pipette tips (1-1000µL)
Stainless steel MALDI target plate (Bruker)

Reagents

Analytical grade water
Ethanol
Formic acid
Acetonitril
Trifluoroacetic acid (TFA)

Work procedure

Culture conditions

1. Culture your organisms under the optimal conditions for each specific species. Make sure to annotate the culture conditions (i.e. growth time, temperature and growth media) under the metadata for each MSP (see point 21).
2. Re-streak a single colony of your species on a fresh culture plate to ensure that there are no contaminations. **USE ONLY FRESH CULTURES TO GENERATE MSPs.**

Sample extraction

3. Perform one Ethanol/formic acid extraction per strain that will be used to generate a new MSP. **NB! This procedure should only be adopted on BSL1, BSL2 or Gram negative BSL3 microorganisms.**
 - a. Add 300µL of analytical grade water into a 1.5mL Eppendorf tube.
 - b. By using a 1µL inoculation loop, pick a loopfull of your organism and dissolve it in the 300µL of water.
 - c. Ensure that the sample is evenly dissolved by vortexing briefly.
 - d. Add 900µL of pure ethanol to each sample and vortex for 1 min.
 - e. Centrifuge the tubes in a table top centrifuge at max speed (13000rpm) for 2 minutes.
 - f. Remove the supernatant and repeat the centrifugation for 10-15 seconds at max speed.
 - g. Remove as much as possible of the ethanol.
 - h. Let the sample air dry (for shorter times leave the tubes open in a fume hood).

- i. Once dry add a volume of 70% formic acid proportional to the amount of biological material transferred in step b (see the table below). Mix thoroughly by pipetting up and down and by vortexing.
- j. Add an equal amount of 100% acetonitrile and mix carefully.

	Small colony	Large colony	1µL inoculation loop
70% Formic acid	1-5µL	1-5µL	10-50µL
100% acetonitrile	1-5µL	1-5µL	10-50µL

- k. Centrifuge the tube for 2 minutes at 13000rpm.
- l. If working in a BSL3 laboratory, spray the tubes with 70% ethanol and put them in a plastic bag before taking it out through the material sluice.
- m. Pipette 1µL of the supernatant onto a clean stainless steel MALDI target and allow the sample to dry (for each strain load 8 spots).
- n. Once dry overlay the spot with 1µL of **FRESH** HCCA matrix solution within 10 minutes.
- o. Allow the sample to dry.

Data analysis

4. Prepare fresh BTS and HCCA matrix and load 2 spots of BTS in the middle of the target plate (e.g. D6, D7)
5. For each strain, load 8 spots per extraction.
6. Wait for the vacuum to sink below $9e^{-7}$ mbar and calibrate the instrument through flex control.
7. Check the intensity for each spot by pressing “run method on current spot” (load autoExecute method: MBT_AutoX). The intensity for each spot has to be over 10 000. If the intensity is not satisfactory, change the laser settings (increase the offset or range by 1 or 2 % under the setup tab) and save this as a new method that will be used on all spots for that sample.
8. Select “new” run and add all the spots to be analyzed in triplicate. For each extraction select a new folder and sample name. TIP! Write the complete MSP name according to the nomenclature described on point 18. under sample name, end by analyzing the BTS once. This BTS is used as a control of the standard curve. Save and start the run by clicking on “start automatic run” .
9. Open flex analysis.
10. First examine the BTS control, open the BTS file and load method >open>MBT_standard.FAMSMETHOD. Perform smoothing and baseline subtraction.
11. Select the BTS spectrum and click on calibrate>internal and then automatic assign. Now check manually that all the peaks are within the following range. **DO NOT click OK.**

Protein	Reference mass (average mass)	±300 ppm range
RL29 [M+2H] ²⁺	3637.8 Da	3636.7 – 3638.8 Da

RS32 [M+H] ⁺	5096.8 Da	5095.3 – 5098.3 Da
RS34 [M+H] ⁺	5381.4 Da	5379.8 – 5383.0 Da
RS33meth [M+H] ⁺	6255.4 Da	6253.5 – 6257.3 Da
RL29 [M+H] ⁺	7274.5 Da	7272.3 – 7276.7 Da
RS19 [M+H] ⁺	10300.1 Da	10297.0 – 10303.2 Da
RNAse A [M+H] ⁺	13683.2 Da	13679.1 – 13687.3 Da
Myoglobin [M+H] ⁺	16952.3 Da	16947.2 – 16957.4 Da

1 or maximum 2 peaks may be outside of these intervals

12. Identify the calibration parameters for BTS by right clicking on the BTS file >properties >calibration. Write down the values for c0, c1 and c2.
13. Close the BTS file without saving it. Open your 24 spectra. Perform smoothing and baseline subtraction on all the spectra. Check that the calibration parameters are the same as for the BTS sample as described under point 12.
14. Zoom in to the spectra and check carefully that there are no outlier spectra (lacking or having an extra peak).
15. For each 1000 m/z region check that all spectra are within a 500 ppm range. By zooming in on one peak (start at 3000 m/z) examine the difference between the spectra with the peak apex furthest to the left and the spectra with the peak apex furthest to the right. This difference must not be more than 500 ppm (e.g. 1.5 m/z for 3000 m/z, 2 m/z for 4000 m/z, 2.5 m/z for 5000 m/z and so on). If a region is lacking a large peak move on to the next region.
16. A maximum of four spectra may be excluded. Otherwise new spectra must be obtained again.
17. Open explorer and change the name of the folder by adding _24, this will allow you to save your raw data and save the modified spectra in a new folder. Back in flex analysis select all the spectra that you have kept and click save.

Generating MSPs

18. Close flex analysis and open Biotyper 3.1. Load your processed spectra, select all, and click action>MSP creation>create. Your new spectrum will be saved under unassigned MSP.

Name your MSP using the following nomenclature: Species name /strain provider or determinant/ strain number / MSP provider (E.g. *Pseudomonas aeruginosa* ATCC 28796 SMI, where ATCC has provided and determined the strain #28796 and SMI has generated the MSP, a clinical isolate determined and provided by SMI will on the other hand be named *Pseudomonas aeruginosa* SMI 28-12 SMI, 28-12 is a unique internal reference number given to distinguish between isolates.

NB! MSPs submitted without the correct nomenclature will be removed.

19. Select this spectrum for identification and run it against the Bruker (or SR) database.
20. Select projects and click on “start taxonomy tree editor” now you can place your spectrum under the desired node.
21. Fill in as much information as you can under MSP metadata. If you don’t have a registered user for provided by or determined by, create one under the Edit MSP metadata window.

22. [Optional] use your spectrum together with the Bruker or SR database to identify the same strain or species from another extraction.
23. Export each MSP **individually** to a folder on your computer and name each MSP file after the MSP name (E.g. Pseudomonas aeruginosa ATCC 28796 SMI will be named Pseudomonas aeruginosa ATCC 28796 SMI.btmsp), this is important because the Spectra database uses the organism name to file the MSPs to the correct organism and the MSP ID (ATCC 28796 SMI) will be automatically added to the New MSPs column in the database.

Upload the MSPs to spectra.folkhalsomyndigheten.se

24. Log in to spectra and open the Bruker database.
25. You can choose to upload single files by selecting the upload button next to the specific organism or you can upload multiple files by selecting the upload multiple files button. The files will automatically be sorted to the correct organism if the organism is present in the database.
26. If the organism is not present in the database you can create an organism by selecting “Add new organism” under the Bruker database tab and fill in the relevant fields. After you have created an organism you can upload your file(s).