Using MALDI to identify two unknown bacteria samples

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Introduction

Matrix assisted laser desorption ionization (MALDI) is a highly sensitive and specific analytical tool used to characterize microbial samples. MALDI is soft ionization technique that utilizes adding a matrix layer over the sample to avoid harsh ionization or heat that can lead to fragmentation or degradation of proteins, peptides or other macromolecules. Yet, this technique is unique to other soft ionization techniques such as Electrospray ionization (ESI) because it is highly sensitive yet more tolerant of salts that are often present on the microbial growth medium, as well as detergents that may form during bacterial growth (1). The sample to be analyzed is spotted onto the metal MALDI test plate (Figure 1) and then mixed with excess matrix. The plate is left to dry, allowing co-crystallization of the matrix and sample spots, before it is placed into the instrument. Inside, the plate is under a vacuum to exclude any air or water. A UV laser beam (typically N2) is then directed to a sample spot on the plate where it irradiates the sample-matrix crystal in short pulses (typically at 337nm). The matrix absorbs the photons emitted by the laser which triggers it's sublimation and ionization into the gas phase which is directly followed by the sublimation and ionization of the sample. These ions enter the time of flight (TOF) tube where they are separated based on their mass to charge ratio *(m/z)*, generating a mass spectrum. Characteristic peaks in a spectra are like a fingerprint for each organism and allows them to be distinguished from one another. The *m/z* ratio will be different for every species, even closely related ones, or subspecies. These characteristic peaks are reference points to that organism in the database that the software can match a given analyte to.

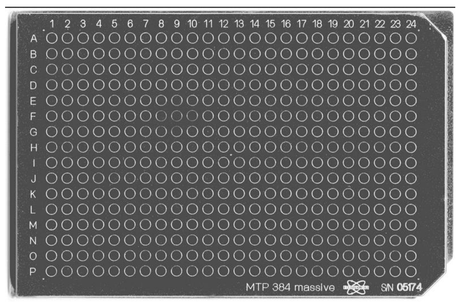

Figure 1. A MALDI target plate. It is composed of metal and is labeled with numbers along the horizontal edge and letters along the vertical edge to facilitate cell identification.

Matrices used in MALDI-TOF MS are generally crystalline solids with low vapor pressure that can easily be volatilized to form ions in a vacuum. Matrices chosen for MALDI share some common characteristics. First, they should be selected for strong absorbance at laser wavelengths to facilitate ionization. It is also critical for a the matrix molecule to have a strong chromophore to allow efficient energy absorption. Second, they must be stable within a vacuum to force the interaction with the sample. Third, the matrix must have an ability to ionize the sample and finally it must completely lack any chemical reactivity with the sample. Some common matrices used to study oligonucleotides, DNA and biopolymers are picolinic acid (PA), 3-hydroxypicolinic acid (HPA) and 3-aminopicolinic acid. Matrices commonly used to analyze proteins and peptides include α-cyano-4-hydroxycinnamic acid (CCA), 2-(-4-hydroxyphenylazo)benzoic acid (HABA) and 2-mercaptobenzothiazole (MBT) (2).

In this experiment, MALDI was used to generate mass spectra of a variety of different bacterial species. Two unknown species were plated and analyzed using MALDI. Based on the characteristic peaks generated due to their specific protein composition, we attempted to identify them by referring to the known bacteria's characteristic peaks.

Experimental

18 different bacterial species were plated on LB agar plates and grown for 48 hours and stored in the fridge for one week prior to analyzing. Each plate that contained bacterial growth was spotted onto a cell on the metal MALDI test plate followed by one microliter of CCA matrix. Two unknowns were also spotted on the MALDI plate by the instructor to analyze.

Results

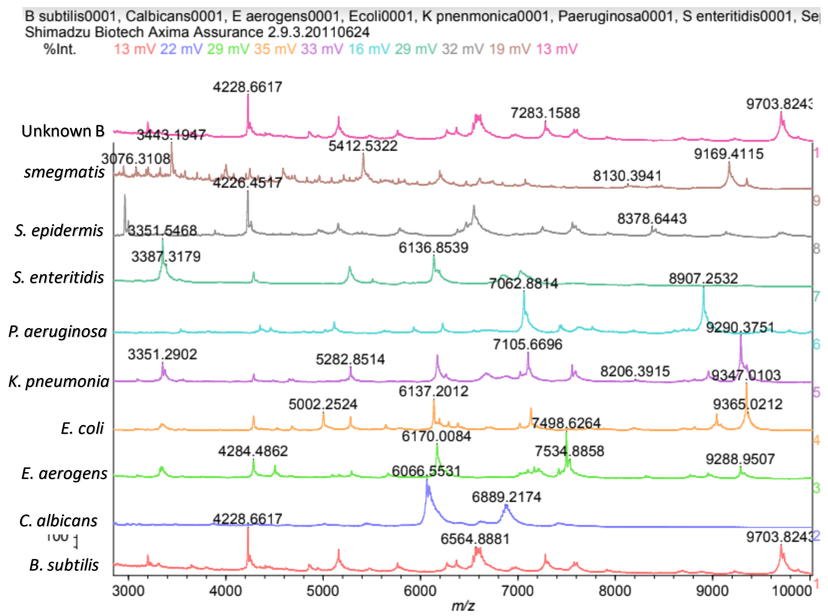

Figure 2. The spectra of all ten bacteria analyzed using MALDI stacked on the same scale. These spectra are compressed and lack some of their characteristic peaks that are more readily observed on a full scale (Figure 3).

Any peaks generated below 1500m/z or above 12000m/z were omitted. A majority of peaks occurred between 4000m/z and 7000m/z. Most spectra did not have peaks that exceeded the mass range of 9000m/z 10000m/z. All spectra contained several highly intense peaks along with less intense ones.

Table 1. Five characteristic peaks in of the bacterial organism's MALDI spectra were identified.

Bacteria species	Characteristic Mass Peaks in Spectra (m/z)
B. subtilis	**2172.2, 4228.7, 5175.6, 6564.9, 7304.6**
C. albicans	1918.9, 4298.2, 6066.6, 6618.2, 6872.1
E. aerogens	1718.6, 4284.5, 6170.0, 7498.6, 9289
E. coli	4285.3, 6137.2, 7133, 9347.0, 10094.5
K. pnenmonica	3351.3, 5282.9, 7105.7, 7557.1, 9290.4
P. aeruginosa	5113.7, 5932.2, 7062.9, 8907.3, 9565.9
S. enteritidis	2777.8, 3351.5, 4286.1, 5271.3, 6136.9
S. epidemis	2963.8, 4226.5, 5787.9, 6549, 7591.0
smegmatis	2706.7, 3443.2, 5412.5, 6198.6, 9169.4
S. aereus	2991.8, 3805.2, 4227.0, 5458.4, 6793.4

Unknown A was determined to be B. subtilis due to the characteristic peaks that the spectra shared (Table 1 and Table 2). Unknown B was not identified as any of the bacteria we analyzed because the characteristic peaks did not match or look similar to any of the bacteria's spectra therefore it could not be identified.

Table 2. Five characteristic peaks from the two unknown spotted samples. Unknown A shows the characteristic peaks as B. subtilis (Table 1). Unknown B does not share any characteristic peaks of the bacteria we analyzed.

Sample	Characteristic Peaks in Spectra
Unknown A	2878.3, 3154.1, 4253.0, 4720.6, 6302.2
Unknown B	2172.2, 4228.7, 5175.6, 6564.9, 7304.6

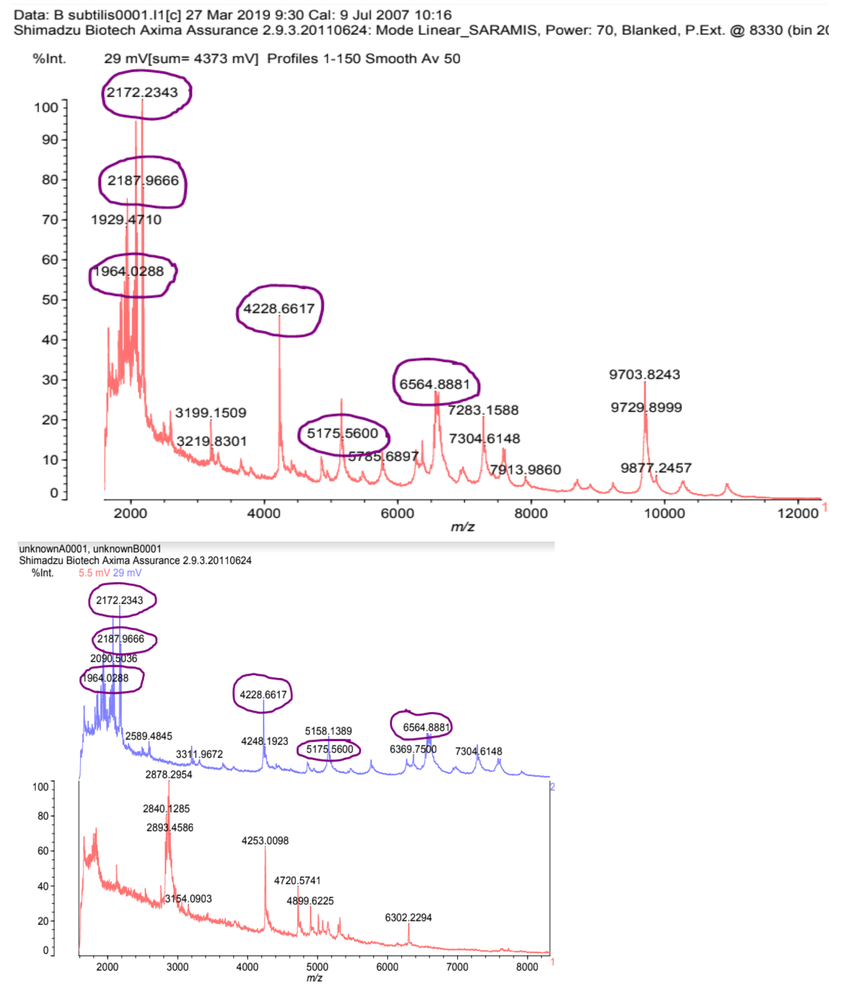

Figure 3. The top spectra is *B. subtilis* which is what we identified Unknown B as. Underneath the full spectra of *B. subtilis* lies the two spectra generated from the unknown samples. Unknown A is on the bottom and unknown B is on top. The characteristic peaks that lead to the identification of Unknown B are circled in the spectra of both Unknown A and *B. subtilis*.

References

(1) Clark, A. E., Kaleta, E. J., Arora, A., & Wolk, D. M. (2013, July). Matrix-assisted laser desorption ionization-time of flight mass spectrometry: A fundamental shift in the routine practice of clinical microbiology. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23824373
(2) Libretexts. (2019, February 23). MALDI-TOF. Retrieved from https://chem.libretexts.org/Core/Analytical_Chemistry/Instrumental_Analysis/Mass_Spectrometry/MALDI-TOF#MatrixProteins