Creation of Custom MALDI-TOF Libraries for Microbiome LBP Consortia Products

Boston Analytical is a leader in the microbiome industry and working with Live Biotherapeutic Products (LBP). Boston Analytical has been leading the industry in cGMP characterization and identification of these LBP products, where the active is a live anaerobic bacterial cell. The Microbiology team at Boston Analytical has been working on developing identification, activity, and purity assays for LBP consortia products.

Microbial identification is a critical assay for microbiome LBP consortia products. However, many LBP consortia production strains are new and novel, and are not always represented in microbial ID databases. By creating a custom library for production strains, microbiome sponsors can create a specialized database that is specific to their proprietary production strains. Custom libraries can be created for Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) instruments, for rapid, proteomic identification. The MALDI-TOF technology allows the Boston Analytical quality control laboratory to quickly identify production strains, without the labor-intensive preparation required for genetic identification. This custom library can be used for identification for release testing, for investigation of environmental monitoring results, or for product breakthrough of production strains during microbial enumeration testing. Boston Analytical can create and maintain these custom libraries, and keep these custom libraries separate to ensure confidentiality.

Pure Culture Isolation and Preparation

Custom libraries are created using well-characterized, defined strains. It is critical to use strain material that has been previously identified using genetic identification and strain typing to ensure, with a high degree of confidence, that the strain material is the intended production organism. The strain material is then sub-cultured on the appropriate agar and incubated at the appropriate temperature. The isolation agar and incubation temperature should be identified during feasibility and validation work for total viable titer testing.

The subculture is incubated for a minimum of 18 hours. At the end of incubation, the subculture is observed to ensure that a uniform culture has been obtained. If non-conforming colonies or potential contaminants are observed, the sub-culturing process is repeated. These non-conforming colonies could potentially contaminate the sample, and create conflicting results.

Once a pure culture has been obtained, the following process is executed:

- Uniform colonies are selected from the plate and added to HPLC-grade water, to create a turbid suspension, and it is vortexed to ensure homogeneity.
- Ethanol is added to the solution, and vortexed again. The suspension is then centrifuged, and the supernatant discarded.
- The pellet is allowed to air-dry and a series of solvents are added to the sample, to release the proteins into solution. The sample is then centrifuged again.
- The resulting supernatant, which now contains the strain-specific proteins that will be used to create the library entry, is plated onto multiple spots of the stainless steel target and allowed to air-dry.
- Bruker HCAA Matrix is plated over the dried sample, and allowed to dry. Once dry, the plate is ready for analysis.

Spectra Analysis and MSP Creation

The sample spots are then analyzed using the MALDI-TOF. The MALDI-TOF uses mass spectrometry to perform proteomic identification of the sample isolate. The HCAA Matrix (as in Matrix Assisted) extracts primarily ribosomal proteins and releases them from the production organism. The laser in the instrument then irradiates the sample with UV light. This focused, intense burst of UV light causes the rapid evaporation of these proteins, releasing positively charged proteins and peptides. The proteins are then electrostatically accelerated and travel up the time of flight tube. A detector in the time of flight tube determines when the proteins arrive. The instrument then measures the amount of time elapsed between the irradiation and the subsequent signal at the detector and determines the molecular mass of the proteins.

These molecular masses are used to create a mass spectrum, which has a mass and intensity distribution pattern that is characteristic of the production strain from which it was created. When performing a routine assay, this spectrum is compared to a reference library to determine if a match is present. For the creation of said reference library, multiple spectra are created by analyzing multiple spots. The spectra are uploaded into Bruker's flexAnalysis software to create a Main Spectra or MSP.

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Using Bruker's flexAnalysis software, the spectra are cleaned up and the baseline removed. The spectra are then compared to the Bacterial Test Standard, to ensure the spectra created meets Bruker validity requirements. After the baseline has been removed and it has been determined that the spectra meet validity requirements, the spectra data set is evaluated in its totality to ensure no outliers are present. These outliers are removed prior to MSP creation, as they are not considered representative of the production strain. The MSP is then created from these selected spectra.

The spectra are uploaded into Bruker's Offline Classification Software to create the MSP. The software combines the spectra into the single Main Spectra. Validity, quality, and library checks are performed on the MSP to make sure it meets requirements before becoming a library entry. The MSP is reviewed to ensure it contains the minimum amount of peaks (>70, per Bruker) and that each peak occurs with the required frequency (at least 25%). The spectra used to create the MSP are then compared to the MSP, to ensure that each spectra matches positively to this new entry. The MSP is also compared to all other library entries to ensure it is unique. Once these checks have been performed, the MSP is ready for validation.

Validation activities are performed, referencing USP Chapter <1113>, "Microbial Characterization, Identification, and Strain Typing." Multiple trials of the validation are performed, leveraging different analysts, strain preparations, and other variables to ensure the routine assay and library entry are robust. The validation, though it is the last stage of the process, is critical to ensuring the library is ready for routine use. Once the validation is complete, the MSP can be uploaded into a new proprietary library or included in an existing library for routine use. The library is now available for release or stability testing.

Microbial Identification is a critical part of the identification process for LBP consortia products, and the creation of a custom, proprietary library on the MALDI-TOF system can greatly improve efficiency. With the quicker pace of the MALDI-TOF assay, efficiencies can be gained during release testing, or with investigation of environmental monitoring, or for production strain breakthrough during microbial enumeration testing. Boston Analytical, working with Bruker, has been able to create custom libraries for proteonomic identification on the MALDI-TOF, to support release and stability testing for LBP consortia products.