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EXPANSION OF MALDI-TOF MS DATABASE FOR IDENTIFICATION OF BACILLUS AND RELATED GENERA FROM MICROBIOTA OF PHARMACEUTICAL CLEAN ROOMS

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RESUMO

Historically, the use of vaccines has been shown to be the most effective way to prevent diseases. During vaccines production process, strict standards must be followed in order to minimize the risk of microbial contamination. To this end, the environmental monitoring program must ensure that productive areas remain within the appropriate levels of control. *Bacillus* and related genera (BRG) are among the main microorganisms isolated from these environments and their elimination is difficult, due to the resistance of the spores. Furthermore, their identification is challenging because of the similarities between closely related species. Most microbial identification systems have databases developed for bacteria of clinical interest, which makes environmental bacteria identification still difficult. Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) is a fast, effective and inexpensive method for microbial identification. However, the databases of this method are still limited for environmental microorganisms. This study aims to expand the database of MALDI-TOF MS to improve identification of BRG from the productive areas of an immunobiological production unit. Ninety-seven strains of BRG, isolated between 2016 and 2017, were initially analyzed by VITEK®MS RUO after 48 h of incubation on blood agar. Then, the strains were identified by 16S rRNA sequencing analysis and phylogenetic tree was constructed with at least 1,203 nucleotides. Then, one representative of each cluster was subjected to analysis of the housekeeping genes *rpoB* and *gyrB*. These strains were incubated in TSA and blood agar for 24, 48 and 72 h and analyzed again by VITEK®MS, so that the spectra obtained were used for the creation of SuperSpectra that were introduced in MALDI-TOF MS database. After improvement of the database, the 97 strains were incubated in TSA and blood agar for 48 h and analyzed again by VITEK® MS. Firstly, the strains were analyzed by VITEK®MS and 77.3% were identified at species/genus level, but 22.7% of the strains were not identified. 16S rRNA gene sequencing analysis provided 94.9% of identifications at species/genus level. The results were inconclusive for five (5.2%) strains and twelve (12.4%) strains were considered possible new species. After 16S rRNA sequencing analysis, 40 profiles were identified. Phylogenetic analysis of the three genes (16S rRNA, *rpob* and *gyrB*) of the 40 selected strains provided the identification of 26 (65.0%) strains at the species level, 12 (30.0%) at the genus level and two (5.0%) were inconclusive. Thirty-two mass spectra were included in the database

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of MALDI-TOF MS. After the database expansion, the percentage of identification by VITEK® MS was improved from 77.3% to 96.9%, overcoming the percentage of identification obtained by genotypic methodology. We concluded that molecular identification by housekeeping genes analysis and the introduction of new spectra provided by MALDI-TOF MS, in order to obtain a customized database, proved to be an extremely promising and effective tool in the identification of BRG from pharmaceutical industry. In addition, this study demonstrated the importance of identifying strains isolated from pharmaceutical production areas to know this microbiota, including the possibility to identify new species.

PALAVRAS-CHAVE: Bacillus, MADI-TOF MS, 16S rRNA

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