

A single-laboratory performance evaluation study of the MALDI-TOF MS system for the identification of *Cronobacter* spp. isolated from food and environmental samples

*Irshad Sulaiman, Nancy Miranda, Whitney Hook, Joseph Mendoza, Steven Simpson, and Kevin Karem
Atlanta Human and Animal Food Laboratory, Office of Regulatory Science, Office of Regulatory Affairs



FDA

Abstract

Cronobacter is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria, that can live in very dry conditions, known to cause life-threatening meningitis and necrotizing enterocolitis in neonates. It is recognized for causing serious illnesses in elderly and immunocompromised individuals as well. Several *Cronobacter* spp. such as *C. sakazakii* have been described as desiccation resistant that can prevail in dry food products. Furthermore, *C. sakazakii* is linked primarily to contaminate powdered infant formula (PIF), and for causing PIF-related sporadic cases and foodborne outbreaks. It has also been isolated from a wide variety of foods worldwide. The VITEK MS is an automated microbial identification system that utilizes novel high-throughput MALDI-TOF mass spectrometry technology. In this single-laboratory performance evaluation study, a VITEK MS-based method was tested for rapid identification of *C. sakazakii*-like strains by analyzing 100 *Cronobacter* spp. strains, recovered from various food and environmental samples (inclusivity), and 30 additional bacterial strains (exclusivity). For species identification of each strain, 1-2 colonies were directly spotted in triplicate; experiment was repeated three times. Our preliminary data analysis suggests that the VITEK MS system could provide genus level identification to all *Cronobacter* spp. strains, and species level identification with high confidence value to most *C. sakazakii* strains examined, under our laboratory condition.

Introduction

- *Cronobacter sakazakii* is a Gram-negative foodborne bacterium known to cause life-threatening meningitis and necrotizing enterocolitis in newborns and immunocompromised individuals.
- It has been linked primarily to contaminate PIF and other related products of public health importance worldwide.
- Of the seven distinctive species of genus *Cronobacter*, three species (*C. sakazakii*, *C. malonaticus*, and *C. turicensis*) have been described as more virulent and recovered frequently from the infant meningitis cases.
- With the innovation of molecular techniques, considerable advancement has been made in the typing of *Cronobacter*.
- Currently, whole genome sequencing (WGS) is applied for precise strain identification of pathogenic bacteria to determine the transmission routes of bacterial diseases and protect consumers from foodborne illness worldwide. However, the major limitation of WGS is that the procedure generates data on a substantial scale. The massive volumes of data generated by the WGS techniques requires additional storage capacity and more time to complete the analysis.
- MALDI-TOF MS technology is widely used for routine microbial identification in clinical, food, environmental, and public health laboratories. This novel tool has been recognized as cost-effective which require shorter time period to complete the analysis, compared to the established DNA-based molecular diagnostic testing methods.
- In this presentation, we report our attempt to complete a study on the potential use of MALDI-TOF MS system for rapid identification of *Cronobacter* spp. isolates recovered from various food and environmental samples.

Methodology

- A total of one hundred *Cronobacter* spp. isolates were recovered from food and environmental surveillance samples following the isolation method described in the FDA's Bacteriological Analytical Manual (BAM).
- Environmental swabs were collected aseptically in the Whirlpak bag (Thermo Fisher Scientific).
- Freshly cultured pure colonies of *Cronobacter* spp. isolates on the TSA (BD) and blood agar (Thermo Fisher Scientific) plates were used to perform MALDI-TOF mass spectrometry analysis.
- The MALDI-TOF MS analysis was performed on a VITEK MS system (bioMérieux).
- For species identification of each isolate, one to two colonies were directly spotted on the manufacturer's proprietary sample plates following the manufacturer's protocols.
- One μ l of CHCA matrix solution (α -cyano-4-hydroxycinnamic acid, bioMérieux) was applied to the samples and air-dried at room temperature for crystallization.
- The MALDI-TOF MS used in this study was equipped with a 337-nm fixed focus nitrogen laser, 50 Hz frequency.
- The mass range between 1-500 kDa produced a single composite mass spectrum.
- *Escherichia coli* ATCC 8739™ isolate was used as the positive control to complete the study.
- The sample spectra were initially analyzed by the VITEK MS IVD analysis software version 3.2 that utilizes a comprehensive propriety clinical database for the identification of bacteria and fungi (bioMérieux).
- Scores between 60% to 100% probability indicated an excellent discrimination value and a reliable identification.
- Score below 60% represented low discrimination identification with a list of choices of possible identification.
- No identification was determined when no match was found for the composite spectra, or not enough spectral peaks were generated during the analysis..

Conclusion

- The VITEK MS system under our laboratory conditions appear to provide rapid genus level identification for the *Cronobacter* spp. isolates.
- It can be used as the first-line method for the identification of the *C. sakazakii* and related strains.

Disclaimer

The findings and conclusions made in this presentation are those of the presenter and do not necessarily represent the views or official position of U.S. Food and Drug Administration (FDA) or the U.S. Department of Health and Human Services (DHHS). The names of vendors or manufacturers are provided as examples of available product sources; it does not imply endorsement of vendors, manufacturers, or products by FDA or DHHS.

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Results and Discussion

- Preliminary analysis of data suggest that the VITEK MS could provide genus-level identification to *Cronobacter* spp. isolates.
- Species-level identification was apparent for approximately >80.0% of the *C. sakazakii*-like isolates with a high confidence value (99.9%) under our laboratory conditions (data not shown).
- The VITEK MS system also identified *C. sakazakii* reference isolate ATCC 29544, and other ATCC reference Gram-negative and Gram-positive bacterial species isolates with a high confidence value (data not shown).
- MALDI-TOF MS is an emerging technique for microbial typing. It has been primarily used in clinical microbiology. However, in recent was it has been applied in the food and environmental microbiology settings.
- While DNA sequencing generates unique DNA fingerprints, MALDI-TOF MS generate unique protein fingerprints by analyzing the whole bacterial cell without involving any extraction and purification process.
- In a previous study, we evaluated and compared the potential of two commercially available MALDI-TOF MS platforms while analyzing ATCC reference isolates of 16 different *Campylobacter* spp. isolates.
- We have utilized this tool for rapid identification of *Staphylococcus aureus* and related species recovered from food, environment, cosmetics, medical device and clinical samples.
- Further, *Lysinibacillus* spp. isolates recovered from cosmetic samples were identified by employing MALDI-TOF MS and 16S rRNA sequencing techniques.
- Species identification was also attained for *Campylobacter*-like isolates recovered from raw poultry products by MALDI-TOF MS and DNA sequencing of 16S rRNA and 23S rRNA regions.
- More recently, various *Clostridium*-like bacterial isolates, recovered from various baby food and baby food supplement surveillance samples, were identified by this tool.
- Failure to attain species identification of a microorganism using a MALDI-TOF MS has been considered to be most likely due to an insufficient protein signal and the absence of an adequate reference spectrum in the database.
- Results for this evaluation study based on three independent experiments to understand the potential of VITEK MS microbial identification system suggests that it is a reliable high-throughput platform that can provide rapid and reproducible genus-level identification for *Cronobacter* spp. isolates under our laboratory conditions.