

SPECIATION OF ENVIRONMENTAL ENTEROCOCCI ISOLATES THROUGH MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time Of Flight) MASS SPECTROMETRY ANALYSIS

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Introduction

In recent years a new approach to identify microorganisms based on mass spectrometric analysis had been proposed. MALDI-TOF MS (Matrix-assisted laser desorption/ionization – Time of Flight Mass Spectrometry) is a powerful method for the detection and identification of proteins by molecular weight determination of specific fragments. Bacterial identification by MALDI-TOF analysis is based on the detection of the mass spectral fingerprint obtained from the whole cell. The method is becoming widely used for the identification of clinically important microorganisms and now this approach is being tested in the environmental microbiology field. The present study aimed to compare the identification accuracy for a fecal indicator (*Enterococcus spp.*) of the biochemical test API® 20 STREP, which is the currently gold standard identification system for Enterococci strains isolated from water samples in our laboratory, with the MALDI-TOF MS technique.

Methods

Water samples from 7 beaches from São Paulo State (Figure 1) were collected during four consecutive weeks and processed by membrane-filtering technique. The filters were incubated on plates of mEI agar for 24 ± 2 h at $41 \pm 0,5^\circ\text{C}$. After incubation presumptive Enterococci colonies were enumerated and the contamination levels were reported as colony forming units (CFU) per 100 mL of water (Method 1600, USEPA, 2006) (Figure 2). Up to five characteristic colonies per sample were randomly selected and preserved for further speciation. A total of 127 bacterial isolates presumptively identified as enterococci on mEI media were speciated both, by the MALDI-TOF MS Bruker Microflex® apparatus (spectra IDs were matched with the Biotyper® Database) and by conventional identification API-Strep system from bioMérieux®.

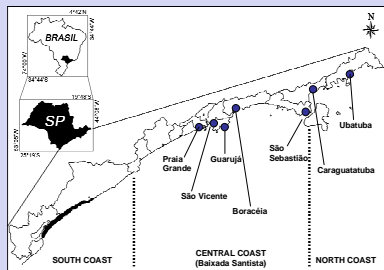


Figure 1. Map of São Paulo State Coast. Blue dots represent the beaches sampled for this study.

Sampled Beaches	Praia Grande	São Vicente	Guarujá	Boracéia	São Sebastião	Caraguatatuba	Ubatuba
Covered period (weeks)	4	4	4	4	4	4	4

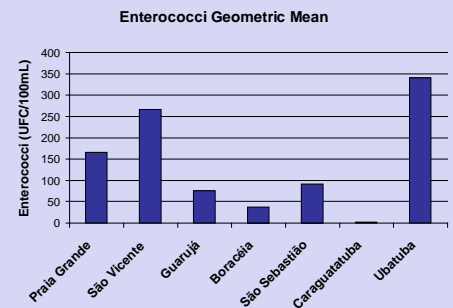


Figure 2. Four-week running Enterococci Geometric Mean.

Results

API® system was able to identify 117 out of 127 strains (92%) whereas MALDI-TOF analysis returned positive results for 100% of the isolates. To the genus level, all the 111 bacterial isolates that were identified as *Enterococcus* by the API test, had the agreement with the MALDI-TOF system. However, to the species level only 63% of the isolates granted the same identification with both systems. API® system identified the 111 *Enterococcus* strains as being 71% *E. faecium*, 12% *E. faecalis* and 17% *E. durans*. API® system did not return results for 4 strains and the remaining 6 strains were identified as being *Aerococcus viridians* (4%) and *Streptococcus bovis* (1%) (Figure 3). By its time, MALDI-TOF analyses generated mass spectra for all isolates and the spectra were matched with Biotyper® 3.0 Software Database for the identification of the microorganisms. 117 out of the 127 isolated were identified belonging to the *Enterococcus* genus with the following distribution: 58% *E. faecium*, 18% *E. faecalis*, 15% *E. hirae*, 5% *E. casseliflavus*, 2,5% *E. gallinarum* and 1% *E. munditti*. Also, MALDI-TOF analyses returned positive results for the other non-enterococci strains which were identified as belonging to *Staphylococcus* and *Streptococcus* genus at the proportions depicted in Figure 4.

API20-STREP Distribution

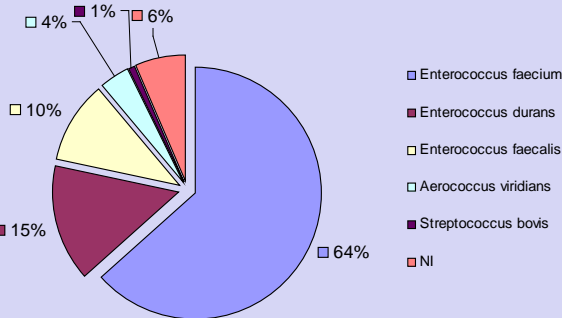


Figure 3. Distribution of the 127 presumptive Enterococci isolates after API20-Strep® speciation.

MALDI-TOF MS Distribution

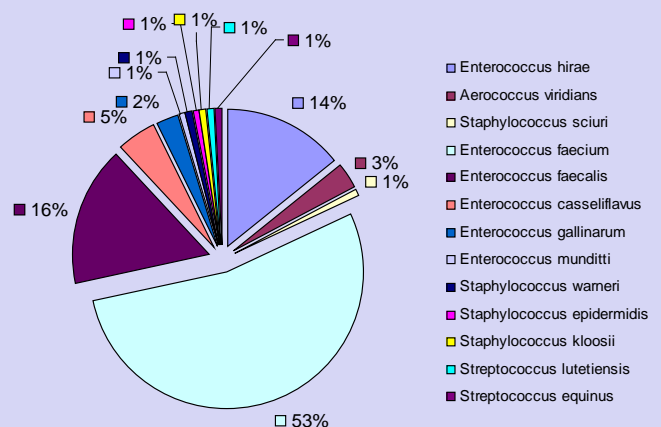


Figure 4. Distribution of the 127 presumptive Enterococci isolates according to the mass spectra matches acquired at the Biotyper® Software Database.

Conclusions

Analysis of presumptive *Enterococci* strains from water samples through MALDI-TOF MS analysis demonstrated to be a valuable tool for quick identification of fecal indicators. Moreover, compared to the well-established Biotyper's API system, MALDI-TOF MS analysis demonstrated a greater resolution to identify *Enterococcus* strains, largely because Bruker's Biotyper® Database shares a broad library of *Enterococci* mass spectra that is continuously validated and fed by laboratories worldwide. Since speciation of *Enterococcus* isolates can differentiate plant-associated from fecal-associated species, this new technique could represent a useful tool for microbial source tracking purposes.

References

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