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Microbial Identification Avian Pathogenic *Escherichia coli* (APEC) Isolated from Poultry by MALDI-TOF MS Method

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A B S T R A C T

To overcome the problems associated with identifying bacteria. The matrix-assisted laser desorption ionization (MALDI) technology was co invented by Franz Hillenkamp and Michael Karas in the mid-1980s. In this work, 77 isolates of avian pathogenic *E. coli* were analyzed by the MALDI-TOF MS method. Total 77 bacterial isolates were identified as *E. coli* on the basis on their mass to charge ratio (m/z). The strain showing ≥1.7 log value with strain in database were confirmed as the member of that genus and strains showing ≥2.0 log values were confirmed as the member of that species. Good quality MALDI-TOF MS spectra were generated for all *E. coli* strains in this study.

Introduction

Bacterial identification has traditionally been a time-consuming and multistep process that included a battery of biochemical tests tailored to each distinct bacteria. To overcome the problems associated with identifying bacteria. Franz Hillenkamp and Michael Karas, two scientists, devised the novel technology and reported soft desorption ionization utilizing an organic compound matrix (Karas and Hillenkamp, 1988), coining the term matrix-assisted laser desorption ionization (MALDI). The extensive use of this technology in clinical microbiology is due to its distinguishing properties, which include an economical, simple, quick, and accurate method for bacterial identification based on automated examination of the mass distribution of bacterial proteins. It significantly reduces identification costs and turnaround times when compared to conventional biochemical and sequencing methods.

The most recent developments regarding MALDI-TOF MS is its regulatory approval for routine bacterial and fungal identification. In this work total 77 isolates of avian pathogenic E. coli were analyzed by the MALDI-TOF MS method. This study demonstrated that MALDI-TOF MS is a reliable fast method for the identification of *E. coli.*

Materials and Methods

This work was carried out at National Centre for Microbial Resource Pune.

Figure.1 MALDI- TOF MS spectrum of representative 10 E. coli isolates of poultry

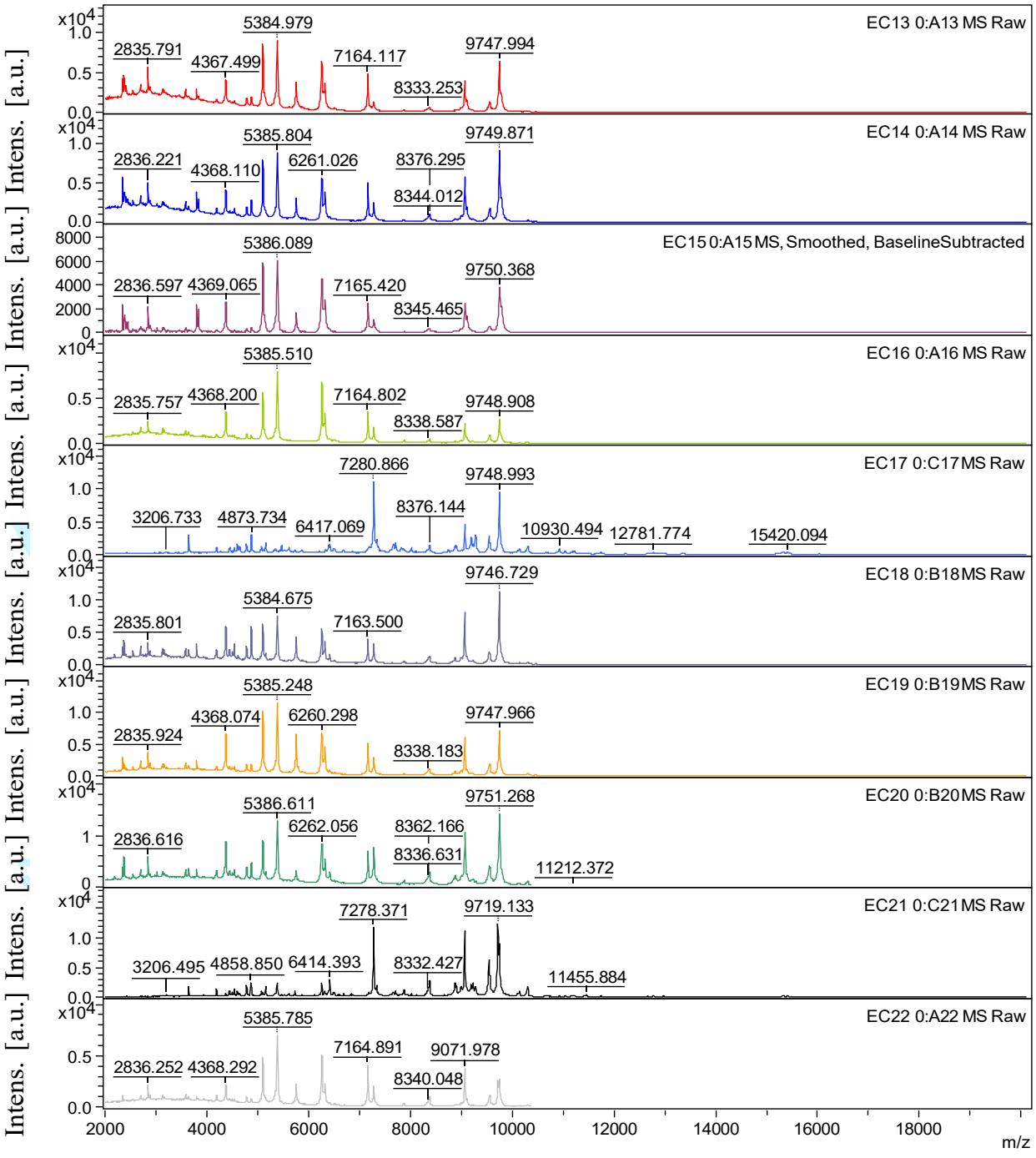

Table.1 MALDI Biotyper identification of *E.coli* isolates

Total no of *E.coli* isolates	MALDI Biotyper score value range results	Interpretation
77 *E.coli* isolates	(score ≥2.0). (N= 77 *E.coli* isolates)	The strains with more than 2.0 score values means reliable species-level identification,

The first step involves mixing of microbial growth from a pure colony with the matrix solution, followed by smear preparation on target plate, air drying, loading of plate inside instrument and finally exposing the samples to source of ionization. After ionization, ionized peptides and proteins travel towards detector in a vacuum tube and they get separated based on their mass to charge ratio (m/z). A mass spectrum of the strain under study is generated, which was then compared with that of the other strains present in the reference database. The database includes biomarkers detected in MALDI spectra of intracellular proteins primarily in the range of 2 to 20 kDa (Fenselau and Demirev, 2001; Rahi *et al.,* 2016). Most of the biomarkers detected in MALDI-TOF spectra of intact bacterial cells have a molecular mass below 15 kDa (Ryzhov and Fenselau, 2001; Croxatto *et al.,* 2012; Suarez *et al.,* 2013). The present investigation was carried out as per method described by Rahi *et al.,* (2016).

Results and Discussion

In this research work total 77 bacterial isolates identified as *E. coli* on the basis on their mass to charge ratio (m/z). The strain showing ≥1.7 log value with strain in database were confirmed as the member of that genus and strains showing ≥2.0 log values were confirmed as the member of that species. Good quality MALDI-TOF MS spectra were generated for all *E.coli* strains. The findings of this research work are in agreement with the findings of Shell *et al.,* (2017). They identified all *E. coli* all and *Salmonella* isolates at the species level (score ≥2.0). Inspection of mass spectra reveals strain-specific peaks at 4375, 5375, 6650, 7190, and 9625 m/z for all *E. coli* isolates 10-20 prominent ion peaks were identified in the mass spectra range. The prominent ion peaks were from the 3000 and 10,500 m/z, with the highest-intensity peaks being in the range of 4375-9625 m/z with *E. coli* isolates. This study demonstrated that Bruker MALDI-TOF MS Biotyper is a reliable fast tool for the identification of *E. coli* (Table 4.9).

In conclusion, the MALDI-TOF MS technique was found to be suitable method for rapid identification of clinically significant *E. coli* isolated from poultry birds affected with Colibacillosis

Author Contributions

D. M. Muglikar: Investigation, formal analysis, writing—original draft. Pravin Rahi: Validation, methodology, writing—reviewing. I. H. Kalyani:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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