Fourth International Conference on Food Science and Technology

Analyzing the effect of culture media composition on Gram-positive bacteria's peptide mass fingerprint

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Introduction

Staphylococcus aureus is one of the most common causes of food poisoning. **Methicillin-resistant *Staphylococcus aureus* (MRSA)** not only found to be the second most common pathogen for deaths related to antimicrobial resistance in recent years, but it is an important pathogen in the community and livestock as well. The importance of *S. aureus* strains is shown in the food production chain that MRSA strains have been documented from wide range of foods sources, such as poultry, pork, beef, dairy products and vegetables. Therefore, the secure and rapid identification of these pathogen is of great importance.

Purpose

The aim of our study was to determine the most suitable culture media to **identify *S. aureus* by MALDI-TOF MS**.

Materials and Methods

The bacterial strains to test culture media's impact on mass spectra were ***S. aureus* ATCC 25923**, a quality control strain for both identification and media testing, and ***S. aureus* ATCC 43300**, a methicillin and oxacillin resistant strain used in susceptibility testing. To identify the isolates, Extended direct transfer procedure was applied (according to Bruker Daltonics), therefore each colony of isolates was placed onto the Bruker's ground steel target plate, overlaid with 1 µL of 70% formic acid and 1 µL of α-cyano-4 hydroxycinnamic acid matrix solution (HCCA). Culture media used in these experiments are **Baird-Parker Agar**, **Reasoner's 2A Agar (R2A)**, **Trypticase Soy Agar (TSA)** and **Yeast Extract Agar**. Discriminant analysis (DA) was used to determine the culture media's impact on the peak list of mass spectra. Both *S. aureus* strains were plated on all culture media. Agar plates were incubated at 37 °C to grow overnight cultures. Both strains were cultivated on the four different culture media in 10 replicates to obtain 80 spectra. One-way ANOVA was applied to compare the identification score values of *S. aureus* isolates cultivated on the four culture media (IBM SPSS Statistics 27). Based on the values of Skewness and Kurtosis, model residuals have normal distribution. Based on Levene's homogeneity of variance was violated ($p<0.001$). ANOVA was significant ($F=22.164$; $p<0.001$), therefore Games-Howell test (Post hoc) was used because of the error variances was violated.

Results and Discussion

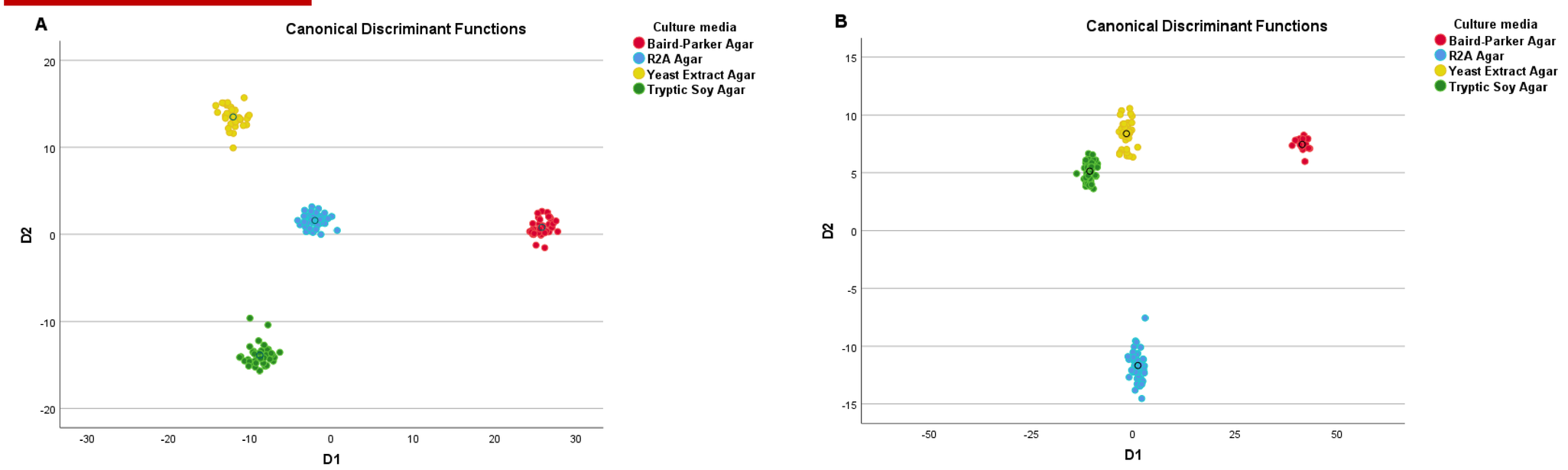

Fig. 1. Discriminant analysis (DA) on the level of culture media's type with the mass spectra of *S. aureus* ATCC 25923 (A) and *S. aureus* ATCC 4300 (B)

Regarding ***S. aureus* ATCC 25923** DA clearly separated Baird-Parker Agar (red) from the rest of the groups which was also visible in identification results, as the lowest scores was achieved on that culture medium. However, a difference can also be noticed in terms of the *S. aureus* strains. In case of *S. aureus* ATCC 25923, groups of TSA (green), R2A (blue) and YEA (yellow) are closer to each other, meaning those spectra are similar to each other. Their identification scores are also closer to each other. As for ***S. aureus* ATCC 43300,** DA shows that the spectra obtained on the two high-nutrient culture media, TSA (green) and Yeast Extract Agar (yellow), are similar to each other. However, not only the group ofBaird-Parker Agar is further away from the two high-nutrient culture media groups but the group of R2A as well.

Table 1 Identification results of MALDI-TOF MS obtained on different culture media

	Baird-Parker Agar		R2A Agar		Yeast Extract Agar		Tryptic Soy Agar	
Bacterial strains	*S. aureus*		*S. aureus*		*S. Aureus*		*S. aureus*	
	ATCC 25923	ATCC 43300	ATCC 25923	ATCC 43300	ATCC 25923	ATCC 43300	ATCC 25923	ATCC 43300
Identification score values	1.92	1.81	2.11	2.14	2.15	2.07	2.16	2.16

Games-Howell post hoc test showed that the average of spectra obtained from Baird-Parker Agar was significantly different from the TSA agar ($p<0.001$). Average of spectra obtained on R2A and Yeast Extract Agar was not differed significantly from the average of TSA agar.

Conclusion

Interestingly, we found that Baird-Parker Agar, the selective and differential culture medium for the isolation and enumeration of *S. aureus* in foods, environmental, and clinical specimens, was the least effective of the tested culture media. Our study demonstrated that R2A and Yeast Extract agars are also suitable to identify *S. aureus* by MALDI-TOF MS as the application of both culture media generated high-confidence identifications. Our study proved that **TSA** can be recommended to identify *S. aureus* by MALDI-TOF MS as the **highest identification score** values were obtained on that culture medium.

References

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