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Application News

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iD^{Plus} Performance/ Food Safety

Identification of Malicious Food-borne Pathogens from Fingerprint Samples using iD^{Plus} Performance

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Introduction

In 2010, World Health Organisation (WHO) revealed that there were almost 582 million food-borne related disease cases which led to about 351 thousand associated deaths worldwide [1]. As food-borne bacteria species are typically transmitted via ingestion of contaminated food, inappropriate handling procedures can threaten the safety of food consumption. Food-borne intoxication can be exceptionally life-threatening, especially for the immunocompromised [2]. In order to reduce the transmission of infectious pathogens, proper hygienic behaviours such as hand washing with water and soap is necessary before handling food materials.

In this experiment, Shimadzu iD^{Plus}, a MALDI-TOF based system, was used to demonstrate the importance of handwashing hygiene through identification of micro-organisms, including various food-borne pathogens, before and after handwashing practices. The approach was based on submitting fingerprint mass peak list from MALDI-TOF mass spectrum to match against SARAMIS database for identification.

Experimental

Individuals were invited to gently imprint five fingerprints from each finger onto a Blood Agar Medium containing Trypticase™ Soy Agar with 5% sheep blood (Thermo Scientific). The bacteria from the fingerprints on the blood agar plate was cultivated at 37°C in an incubator (MCO-5AIC, Sanyo) with 5% CO_2 for 18 to 48 hours. After which, different single bacteria colony was chosen from the plates and transferred onto another blood agar plate for pure bacteria sub culturing (Figure 1).

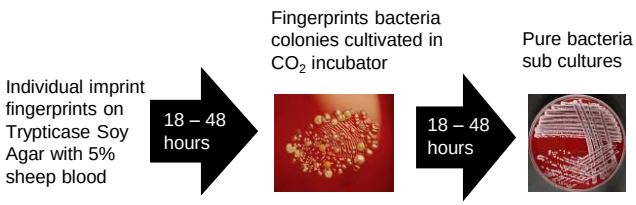

Figure 1: Flow chart of bacteria culture

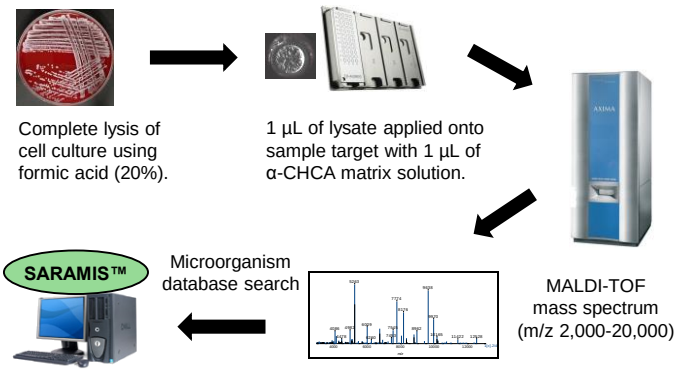

Figure 2: Workflow of iD^{Plus} platform

The workflow of iD^{Plus} platform (Figure 2) consists of three steps: sample preparation, mass spectrometry measurement and identification by SARAMIS. Individual colony was taken from the agar plate and lysed in 20 µL of 20% formic acid. An amount of 1 µl of lysate was applied onto a stainless steel MALDI target followed by 1 µL of matrix solution (10 mg/ml of a-cyano-4-hydroxy-cinnamic acid in acetonitrile : ethanol : water (1:1:1) with 0.3 % trifluoro acetic acid). The mixture was then allowed to dry at room temperature leading to a co-crystallization of matrix and analyte molecules. Mass spectrometry measurements were performed on MALDI-TOF MS (AXIMA Performance, Shimadzu Corporation). Mass spectra were acquired in positive linear mode (m/z=2,000-20,000) using an acceleration voltage of 20 kV and a low mass gate of 1,500 Da. *E. coli* DH5α Electro-cell (TaKaRa bio Inc., Japan) was used as standard for mass calibration. The identification was performed by comparing the sample mass spectra against SuperSpectra in the SARAMIS database.

Results and Discussion

Fourteen different types of micro-organisms were found on the fingerprint samples of individuals before hand washing. Most of them can be found in the normal human microbiota or naturally from the environment (see Table 1).

Among them, ***Bacillus cereus*** and ***Staphylococcus aureus***, were found on the fingerprint samples obtained from unwashed hands. They are food-borne pathogens, as listed by the Centers for Disease Control and Prevention [3]. These bacteria species can be easily transferred when infected individuals handle or consume food with their bare hands.

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Table 1: Microorganism identification results from individuals' fingerprints before hand washing by iD^{Plus} platform

Name	%	Family	Genus	Species
HA4-3	99.90	Family I Micrococceae	Micrococcus	luteus
HA1	99.90	Family VIII Staphylococcaceae	Staphylococcus	warneri
LB2-1	90.00	Family VIII Staphylococcaceae	Staphylococcus	capitis
LB1-8	**99.90**	**Family VIII Staphylococcaceae**	**Staphylococcus**	**aureus**
SA4	99.90	Family VIII Staphylococcaceae	Staphylococcus	haemolyticus
SB3-7	99.90	Family VIII Staphylococcaceae	Staphylococcus	epidermidis
HB1-2	**99.90**	**Family I Bacillaceae**	**Bacillus**	**cereus group**
SB2	99.90	Family VIII Staphylococcaceae	Staphylococcus	hominis
2A1	99.90	Family I Bacillaceae	Bacillus	subtilis
2A2	99.90	Family I Enterobacteriaceae	Proteus	mirabilis
X1	99.90	Family I Neisseriaceae	Neisseria	subflava
YNHM-4	93.20	Family VIII Staphylococcaceae	Staphylococcus	cohnii
TGNH-4	99.90	Family I Rhodobacteraceae	Paracoccus	yeei
E-HG-4	99.90	Family VI Streptococcaceae	Streptococcus	parasanguinis

After hand washing, it was discovered that the pathogens were no longer found from their fingerprint samples with washed fingers. Figures 3a and 3b show the mass spectrum and cell cultures of the ***Bacillus cereus*** (a) and ***Staphylococcus aureus*** (b).

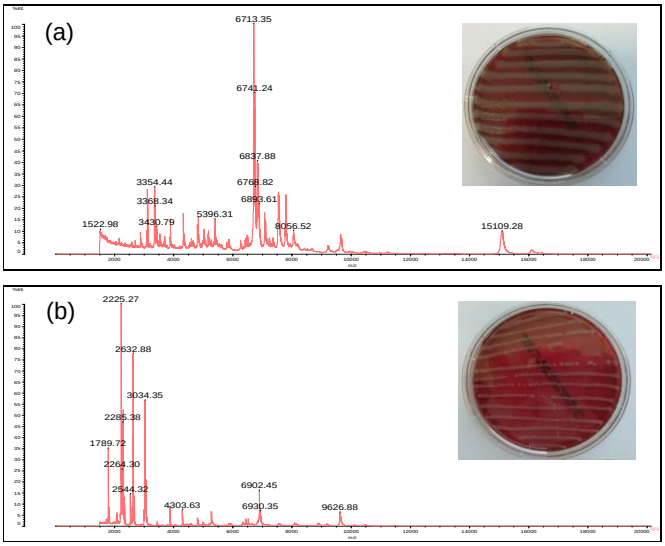

Figure 3: MS spectrum and cell culture of ***Bacillus cereus*** (a) and ***Staphylococcus aureus*** (b)

Table 2 shows the reproducibility and reliability identification results of ***Bacillus cereus*** and ***Staphylococcus aureus*** on the iD^{Plus} quick micro-organism identification platform. Each culture sample was prepared and analysed in 6 wells on

Table 2: Identification results of ***Bacillus cereus*** and ***Staphylococcus aureus*** by iD^{Plus} platform

Name	%	Family	Genus	Species
HB1-7	99.9	Family I Bacillaceae	Bacillus	cereus group
HB1-6	99.9	Family I Bacillaceae	Bacillus	cereus group
HB1-5	99.9	Family I Bacillaceae	Bacillus	cereus group
HB1-4	99.9	Family I Bacillaceae	Bacillus	cereus group
HB1-3	99.9	Family I Bacillaceae	Bacillus	cereus group
HB1-2	99.9	Family I Bacillaceae	Bacillus	cereus group
LB1-8	99.9	Family VIII Staphylococcaceae	Staphylococcus	aureus
LB1-6	99.9	Family VIII Staphylococcaceae	Staphylococcus	aureus
LB1-5	98.9	Family VIII Staphylococcaceae	Staphylococcus	aureus
LB1-4	99.9	Family VIII Staphylococcaceae	Staphylococcus	aureus
LB1-3	98.9	Family VIII Staphylococcaceae	Staphylococcus	aureus
LB1-2	99.9	Family VIII Staphylococcaceae	Staphylococcus	aureus

MALDI target, all of which yielded identification results with more than 90.0% (threshold for identification) confidence level by SuperSpectra.

SuperSpectra are artificial fingerprint mass spectra generated based on reference spectra of well-known microorganisms, which comprises of genus, species and strain-specific protein fingerprints [4]. The specificity of protein fingerprints is established by comparing multiple spectra of isolates of the same species, genus, and family, where each peak in a SuperSpectrum receives a peak weight corresponding to its specificity. This means that for species identification, all peaks that are specific only on the genus or family level receive a low peak weight – dimensionless points - while species-specific peaks are accentuated. Each SuperSpectra is computed from mass spectral data of at least 15 different representatives (isolates) of one species from different locations (hospitals, reference centers and culture collections), where each species is characterised by one or more alternative acknowledged procedures, e.g. biochemical methods, 16sRNA-sequencing. This ensures credible identifications with no false positive results.

Conclusions

The iD^{Plus} utilises SARAMIS to rapidly and reliably determine micro-organism on unwashed hands, including various food-borne pathogens and also its absence after handwashing practices. Proper food handling and sanitation practices are indeed crucial in reducing the risk of transmitting infectious pathogens among humans.

Note: For research use only, Not for use in diagnostic procedures.

References

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