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The Application of MALDI-TOF MS for Identification of Bacteria from Consumer Goods Industries

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Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized microorganism identification. Studies have applied this technology for identification of clinical isolates and verified its speed, high-throughput and cost effectiveness. Species from industries might be different with those of clinical isolates, and identifications of industrial bacteria are rarely reported. In this study, we applied this technology for industrial bacteria identification. We collected 152 strains from consumer goods industries, which scored lower than 2.0 (unreliable at species level). The strains were further analyzed by 16S rRNA and housekeeping gene sequence analysis, and physiological and biochemical analysis where necessary. The accuracy of MALDI-TOF MS identification highly depends on the scale of the spectra database. By enriching the database with the obtained mass spectrometry data (spectra and identifications), industrial isolate identifications can be improved by MALDI-TOF MS. This will enhance the robustness of this system beyond its current exceptional performance that includes use for in vitro diagnostics.

Keywords: MALDI-TOF MS; Consumer goods; Bacteria; Identification.

Bacterial identification is routinely carried out by 16S rRNA gene sequence analysis, supplemented with housekeeping gene sequence analysis and phenotypic characteristics such as colony morphology, microbial physiology and biochemical analysis[14]. Recently, bacterial isolates have also been identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which offers a time-saving, high throughput and cost effective alternative[5, 22]. MALDI-TOF MS has been routinely used since the mid-1990s and was later verified in the identification of clinical microbial isolates[7, 9, 13, 28, 31]. Unlike molecular biological methods such as fluorescence in situ hybridization (FISH) and real-time polymerase chain reaction (RT-PCR), MALDI-TOF MS requires no species-specific probes, considerably reducing the identification cost[11, 29].

Most studies adopting MALDI-TOF MS for bacterial identification have focused on clinical bacterial isolates[9]. Food, cosmetic and similar industries also require fast, stable and accurate microbial identification methods. However, industrial applications of MALDI-TOF MS, such as the differentiation of industrial *Saccharomyces cerevisiae* strains, are rarely reported[19]. Therefore,

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to better meet the requirements of industrial microbial identification, an efficient way is to accumulate more information on industrial isolates in the spectrum database. We applied MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA) for identification of hundreds of strains from fast-moving consumer goods (FMCG) industries, and found 152 strains with scores lower than 2.0, which were recognized as not reliable at species level. In this study, the 152 strains were further analyzed by the reference methods, including molecular biological analysis (16S rRNA gene sequence analysis and housekeeping gene sequence analysis), and physiological and biochemical analysis if necessary. The MALDI-TOF MS results were paralleled with those of the reference method. Data supplementation would significantly expand the identification accuracy and applicability of MALDI-TOF MS and facilitate its use in the FMCG industries.

MATERIALS AND METHODS

Bacterial strains

This study analyzed 152 bacterial strains. Twenty-seven strains were purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). DSMZ strains were collected according to the common bacteria related to FMCG industries counted by CICC (China Center of Industrial Culture Collection, Beijing, China), and not present in the commercial Bruker BDAL Biotyper database. The rest 125 strains, whose MALDI-TOF MS scores were lower than 2.0, were isolated from FMCG industries by CICC.

MALDI-TOF MS operation and identification

The isolates were recovered on TSA plates, and single colonies were picked for mass spectral measurements using a Bruker Microflex LT MALDI-TOF MS platform with the Bruker BDAL MSP database (Bruker Daltonics, Billerica, MA, USA) linked to FlexControl software (version 4.0). The colonies were prepared according to the manufacturers' formic acid extraction instructions. Briefly, one colony was picked using a sterile disposable plastic loop and mixed with 1.2 ml of 75% ethanol (v/v) in an Eppendorf tube. The mixture was centrifuged at 13,000 × *g* for 2 min and the supernatant was discarded. The pellet was resuspended in 50 ìl of 70% formic acid and 50 ìl acetonitrile, and again centrifuged at 13,000 × *g* for 2 min. One microliter of the supernatant was pipetted onto the sample loading spot on the stainless steel target plate, and covered with a 1 ìl-portioned alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics, Billerica, MA, USA). Up to 240 spectra per isolate were generated in positive ion linear detection mode. The summed spectra were imported into the integrated MALDI Biotyper software (version 4.0) and analyzed by standard pattern matching with manufacturers' default settings. The spectrum of each isolate was searched in the Biotyper BDAL MSP database, and a reliability score was generated from the similarity measure. Scores were interpreted as not reliable (< 1.7), reliable at the genus level (1.7 d" score < 2.0), or reliable at the species level (e" 2.0). Each colony was analyzed at least 4 times.

One bottle of Bacterial Test Standard (BTS, 40 standard spots, Bruker Daltonics, Billerica, MA, USA) was suspended in 50 ìl of premixed solvent (50% acetonitrile and 2.5% trifluoroacetic acid). As positive and negative controls in each run, we used 1 ìl of the BTS solution (prepared for loading as stated above) and 1 ìl of HCCA matrix alone. All chemicals were purchased from Sigma–Aldrich (Steinheim, Germany), unless stated otherwise. The standard protocol used in the work yielded quality spectra with good resolution and spectral information, and the BTS results verifying correct operation of the system for all samples analyzed.

16S rRNA gene sequence analysis

The genome DNA of the bacteria was extracted using a Bacterial Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China). The 16S rRNA gene was amplified by the primers 27f and 1492r [15] (Table S1). The PCR products of all tested strains were sequenced in an ABI 3730 DNA analyzer system (ABI, USA). All of the nucleotide sequences were aligned by EzBiocloud (http://ezbiocloud.net/) and searched in the NCBI database using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast).

Housekeeping gene sequence analysis

The housekeeping genes *atpD*[4], *gyrA*[6], *gyrB*[27], *rpoB* [1] and *tuf* [2] were amplified by PCR and sequenced for strain identification. The analysis was identical to the 16S rRNA gene sequence analysis. The amplification primer pairs for each gene are listed in Table S1.

Physiological and biochemical analysis

Some strains that could not be identified to species level were physiologically and biochemically characterized. The characterizations were performed manually or in an API system (BioMérieux, Paris, France).

RESULTS

Hundreds of strains related to FMCG were analyzed by MALDI-TOF MS, and many of them were well identified. However, 152 strains scored lower than 2.0 were found indicating that these strains were recognized as not reliable at species level. Among these were 78 strains with scores

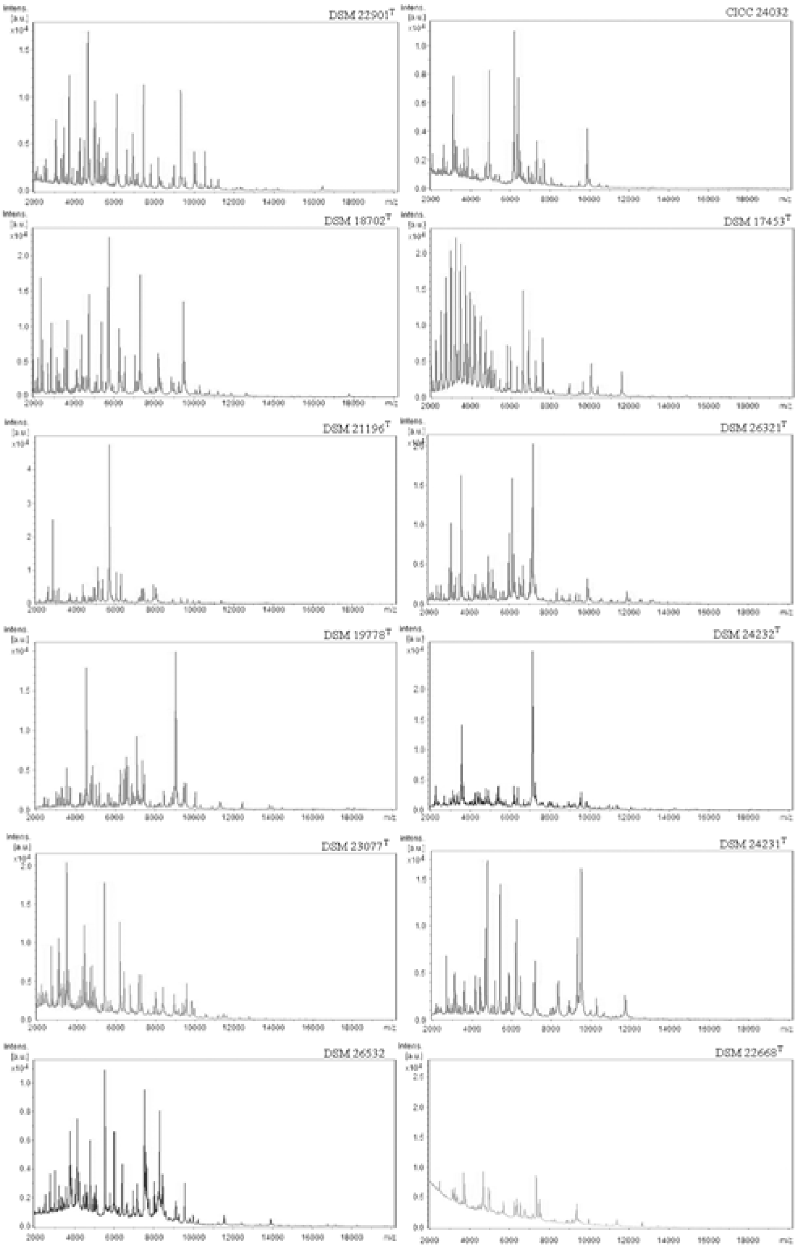

Fig. 1. The frequency of occurrence of the 152 strains

Table 1. Strains did not include in the MS database with MALDI-TOF MS scores < 1.7

Strain No.	Genus/Family (Strain number)	16S rRNA gene sequencing		MALDI-TOF MS		Remark
		Species ID	Level of ID	Species ID	Score	
CICC 23979	*Acinetobacter* (2)	*Acinetobacter bereziniae*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis
DSM 25388 ^{T}		*Acinetobacter indicus*	Species	-	< 1.700	
CICC 24053	*Aeromonas* (1)	*Aeromonas* sp.	Genus	-	< 1.700	New species
CICC 24062	*Anaerococcus* (1)	*Anaerococcus* sp.	Genus	-	< 1.700	New species
DSM 15972 ^{T}	*Asaia* (1)	*Asaia siamensis*	Species	-	< 1.700	MS database did not contain the genus
CICC 10075	*Bacillus* (13)	*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens*	Species	-	< 1.700	Housekeeping gene (*gyrA*) analysis
CICC 10079		*Bacillus amyloliquefaciens* subsp. *amyloliquefaciens*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 20037		*Bacillus amyloliquefaciens* subsp. *plantarum*	Species	-	< 1.700	Housekeeping gene (*gyrA*) analysis
CICC 10265		*Bacillus amyloliquefaciens* subsp. *plantarum*	Species	-	< 1.700	Housekeeping gene (*gyrA*) analysis
CICC 23981		*Bacillus amyloliquefaciens* subsp. *plantarum*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 23985		*Bacillus amyloliquefaciens* subsp. *plantarum*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 23987		*Bacillus atrophaeus*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 23978		*Bacillus halosaccharovorans* or *Bacillus niabensis*	Two different species possible	-	< 1.700	
CICC 24021		*Bacillus halosaccharovorans* or *Bacillus niabensis*	Two different species possible	-	< 1.700	
DSM 28326 ^{T}		*Bacillus methylotrophicus*	Species	-	< 1.700	
DSM 24771 ^{T}		*Bacillus oceanisediminis*	Species	-	< 1.700	
CICC 24027		*Bacillus pumilus*	Species	-	< 1.700	
CICC 24023		*Bacillus subtilis*	Species	-	< 1.700	

DSM 11554^T	*Bradyrhizobium* (1)	*Bradyrhizobium elkanii*	Species	-	< 1.700	
DSM 22706^T	*Burkholderia* (1)	*Burkholderia contaminans*	Species	-	< 1.700	
CICC 24037	Comamonadaceae (1)	Comamonadaceae	Family	-	< 1.700	New species
CICC 23986	*Corynebacterium* (1)	*Corynebacterium* sp.	Genus	-	< 1.700	New species
CICC 20570	*Delftia* (1)	*Delftia lacustris*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 23951	*Dermacoccus* (1)	*Dermacoccus profundi*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 24057	*Domibacillus* (1)	*Domibacillus robiginosus*	Species	-	< 1.700	MS database did not contain the genus
CICC 23781	*Exiguobacterium* (1)	*Exiguobacterium* sp.	Genus	-	< 1.700	
CICC 11014s	*Halomonas* (7)	*Halomonas johnsoniae*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 11015s		*Halomonas johnsoniae*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 11016s		*Halomonas johnsoniae*	Species	-	< 1.700	Physiological and biochemical analysis
DSM 15367^T		*Halomonas magadiensis*	Species	-	< 1.700	
CICC 11012s		*Halomonas* sp.	Genus	-	< 1.700	New species; Housekeeping gene (*gyrB*) analysis
CICC 11013s		*Halomonas stevensii*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 11011s		*Halomonas* sp.	Genus	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 24029	*Kocuria* (2)	*Kocuria flava*	Species	-	< 1.700	
CICC 24030		*Kocuria marina*	Species	-	< 1.700	
CICC 21102	*Komagataeibacter* (1)	*Komagataeibacter saccharivorans*	Species	-	< 1.700	Housekeeping gene (*rpoB*) analysis; MS database did not contain the genus
CICC 23996	*Kytococcus* (1)	*Kytococcus aerolatus*	Species	-	< 1.700	
CICC 10774	*Lactobacillus* (1)	*Lactobacillus acetotolerans*	Species	-	< 1.700	
CICC 24005	*Luteococcus* (1)	*Luteococcus peritonei*	Species	-	< 1.700	MS database did not contain the genus
CICC 23595	*Lysinibacillus* (6)	*Lysinibacillus macroides*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 23596		*Lysinibacillus macroides*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 20858		*Lysinibacillus xylanilyticus*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 10600		*Lysinibacillus* sp.	Genus	-	< 1.700	
CICC 10601		*Lysinibacillus* sp.	Genus	-	< 1.700	
CICC 10602		*Lysinibacillus* sp.	Genus	-	< 1.700	
CICC 23971	*Massilia* (2)	*Massilia suwonensis*	Species	-	< 1.700	
DSM 21873^T		*Massilia varians*	Species	-	< 1.700	
DSM 16372^T	*Methylobacterium* (1)	*Methylobacterium hispanicum*	Species	-	< 1.700	
CICC 24058	*Microbacterium* (1)	*Microbacterium esteraromaticum* or *Microbacterium*	Two different species possible	-	< 1.700	

		arabinogalactanolyticum				
CICC 23969	*Mycobacterium* (2)	*Mycobacterium phocaicum*	Species	-	< 1.700	Housekeeping gene (*rpoB*) analysis
CICC 23970		*Mycobacterium phocaicum*	Species	-	< 1.700	Housekeeping gene (*rpoB*) analysis
CICC 24049	*Novosphingobium* (2)	*Novosphingobium panipatense* or *Novosphingobium mathurense*	Two different species possible	-	< 1.700	
CICC 11035s		*Novosphingobium* sp.	Genus	-	< 1.700	New species
CICC 24010	*Oceanobacillus* (1)	*Oceanobacillus kimchii*	Species	-	< 1.700	Physiological and biochemical analysis; MS database did not contain the genus
CICC 23975	*Paenibacillus* (2)	*Paenibacillus humicus*	Species	-	< 1.700	
CICC 24054		*Paenibacillus hunanensis*	Species	-	< 1.700	
CICC 24025	*Pantoea* (1)	*Pantoea* sp.	Genus	-	< 1.700	New species
CICC 23980	*Planomicrobium* (1)	*Planomicrobium* sp.	Genus	-	< 1.700	MS database did not contain the genus
DSM 22348^{T}	*Pseudomonas* (5)	*Pseudomonas japonica*	Species	-	< 1.700	
CICC 24007		*Pseudomonas kuykendallii*	Species	-	< 1.700	
CICC 24008		*Pseudomonas kuykendallii*	Species	-	< 1.700	
CICC 24042		*Pseudomonas* sp.	Genus	-	< 1.700	New species
CICC 24061		*Pseudomonas* sp.	Genus	-	< 1.700	New species
CICC 24046	*Pseudoxanthomonas* (2)	*Pseudoxanthomonas* sp.	Genus	-	< 1.700	New species
CICC 24048		*Pseudoxanthomonas* sp.	Genus	-	< 1.700	New species
DSM 14664^{T}	*Psychrobacter*(3)	*Psychrobacter faecalis*	Species	-	< 1.700	
CICC 24001		*Psychrobacter faecalis* or *Psychrobacter pulmonis*	Two different species possible	-	< 1.700	Housekeeping gene (*gyrB*) analysis
DSM 23635^{T}		*Psychrobacter sanguinis*	Species	-	< 1.700	
CICC 23973	*Rhizobium* (1)	*Rhizobium larrymoorei*	Species	-	< 1.700	
CICC 24028	*Roseomonas* (1)	*Roseomonas aestuarii*	Species	-	< 1.700	
DSM 7462^{T}	*Sphingobium* (1)	*Sphingobium yanoikuyae*	Species	-	< 1.700	
DSM 15593^{T}	*Sphingomonas* (1)	*Sphingomonas roseiflava*	Species	-	< 1.700	
DSM 13593^{T}	*Sphingopyxis* (1)	*Sphingopyxis alaskensis*	Species	-	< 1.700	
DSM 23150^{T}	*Sporosarcina* (1)	*Sporosarcina luteola*	Species	-	< 1.700	
CICC 23995	Staphylococcaceae (1)	Staphylococcaceae	Family	-	< 1.700	New species
CICC 23946	*Xanthobacter* (1)	*Xanthobacter flavus*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 10225	*Zymomonas* (2)	*Zymomonas mobilis*	Species	-	< 1.700	MS database did not contain the genus
CICC 41465		*Zymomonas mobilis* subsp. *mobilis*	Species	-	< 1.700	Housekeeping gene (*gyrB*) analysis; MS database did not contain the genus

Table 2. Strains did not include in the MS database with MALDI-TOF MS scores ≤ 1.7.

Strain No.	Genus (Strain number)	16S rRNA gene sequencing Species ID	MALDI-TOF MS Level of ID	Remark Species ID	Score
DSM 22901^{T}	*Acinetobacter* (1)	*Acinetobacter beijerinckii*	Species	*Acinetobacter tjernbergiae*	1.816 ± 0.077
CICC 24032	*Bacillus* (1)	*Bacillus subtilis*	Species	*Bacillus amyloliquefaciens*	1.742 ± 0.022
DSM 18702 ^{T}	*Cronobacter* (1)	*Cronobacter malonaticus*	Species	*Cronobacter sakazakii*	2.099 ± 0.180
DSM 17453 ^{T}	*Chryseobacterium* (1)	*Chryseobacterium taichungense*	Species	*Chryseobacterium hagamense*	1.789 ± 0.016
				Chryseobacterium sp.	1.811 ± 0.067
DSM 21196 ^{T}	*Halomonas* (1)	*Halomonas hamiltonii*	Species	*Halomonas aquamarina*	1.788 ± 0.062
DSM 26321 ^{T}	*Massilia* (1)	*Massilia oculi*	Species	*Massilia timonae*	1.930 ± 0.137
DSM 19778 ^{T}	*Ochrobactrum* (1)	*Ochrobactrum cytisi*	Species	*Ochrobactrum* sp.	2.121 ± 0.185
				Ochrobactrum tritici	2.047 ± 0.095
DSM 24232 ^{T}	*Pantoea* (3)	*Pantoea brenneri*	Species	*Pantoea agglomerans*	1.753 ± 0.032
DSM 23077 ^{T}		*Pantoea eucalypti*	Species	*Pantoea agglomerans*	1.819 ± 0.085
DSM 24231 ^{T}		*Pantoea eucrina*	Species	*Providencia rettgeri*	1.765 ± 0.038
DSM 26532	*Pseudomonas* (1)	*Pseudomonas baetica*	Species	*Pseudomonas koreensis*	1.721 ± 0.011
DSM 22668^{T}	*Rhizobium* (1)	*Rhizobium pusense*	Species	*Rhizobium radiobacter*	1.965 ± 0.106

below 1.7 indicating no reliable identification by MALDI-TOF MS (Table 1). The mass spectra and associated identifications of the 78 strains can be added to expand the MS database for industrial strains identification.

Twelve of these strains yielded different identifications by molecular biological methods and the MALDI-TOF MS analysis (Table 3). For some strains, the scores ranged from 1.7 to greater than 2.0, suggesting low repeatability of MALDI-TOF MS for these strains. The MALDI-TOF MS results and molecular biological identifications of these strains were consistent at the genus level, but mismatched at the species level. The instrument parameters and sample preparation were all carried out by standard protocol, and generated qualified mass spectra (Fig. S1). Therefore, such discrepancies reflect the absence of these species in the MALDI-TOF MS database rather than inaccuracy of MALDI-TOF MS, and highlight the necessity and importance of supplementing the MS database.

Forty-eight of the 152 strains were listed in the MALDI-TOF MS database, of which 21 scored below 1.7 in the MALDI-TOF MS analysis (Table 4). These species were successfully identified by gene sequence and physiological/ biochemical analyses, and found in the MALDI-TOF MS database. Highly qualified mass spectra data were obtained based on standard protocol (Fig. S2). The MALDI-TOF MS failure was possibly caused by metabolic and psychological divergence between the present isolates and the bacteria or type strains archived in the database[24].

The remaining strains, scoring between 1.7 and 2.0, are listed in Table 5. The strain labeled CICC 24011 was molecularly identified as *Acinetobacter parvus* but as *A. junii* by MALDI-TOF MS. As *A. parvus* and *A. junii* have not been compared in previous reports, the reason for this misidentification could be sought in future study. The sample strains labeled CICC 23959, CICC 23967, CICC 24012, CICC 24013, CICC 24019, CICC 24024, CICC 24031, CICC 24033, CICC 24036, and CICC 24059 were identified to genus level by molecular biological method and consistent with the results of MALDI-TOF MS. The rest of the strains were identified to species level by molecular biological method while reliable only at the genus level by MALDI-TOF MS, possibly because of the

Table 3. Strains included in the MS database with MALDI-TOF MS scores < 1.7

Strain No.	Genus (Strain number)	16S rRNA gene sequencing		MALDI-TOF MS		Remark
		Species ID	Level of ID	Species ID	Score	
CICC 23998	*Bacillus* (7)	*Bacillus circulans*	Species	-	< 1.700	
CICC 23977		*Bacillus infantis*	Species	-	< 1.700	
CICC 23972		*Bacillus licheniformis*	Species	-	< 1.700	Housekeeping gene (*gyrA*) analysis
CICC 23974		*Bacillus licheniformis*	Species	-	< 1.700	Housekeeping gene (*gyrA*) analysis
CICC 24016		*Bacillus endophyticus*	Species	-	< 1.700	
CICC 24017		*Bacillus subtilis*	Species	-	< 1.700	
CICC 24034		*Bacillus subtilis*	Species	-	< 1.700	
CICC 23966	*Brevibacterium* (1)	*Brevibacterium sanguinis* or *Brevibacterium celere*	Two different species possible	-	< 1.700	
CICC 24026	*Curtobacterium* (1)	*Curtobacterium* sp.	Genus	-	< 1.700	
CICC 24018	*Dermacoccus* (1)	*Dermacoccus* sp.	Genus	-	< 1.700	
CICC 24060	*Exiguobacterium* (3)	*Exiguobacterium* sp.	Genus	-	< 1.700	
CICC 24040		*Exiguobacterium* sp.	Genus	-	< 1.700	
CICC 24022		*Exiguobacterium* sp.	Genus	-	< 1.700	
CICC 23952	*Kocuria* (2)	*Kocuria carniphila*	Species	-	< 1.700	Physiological and biochemical analysis
CICC 23997		*Kocuria rosea* or *Kocuria polaris* or *Kocuria himachalensis*	Three different species possible	-	< 1.700	
CICC 23990	*Micrococcus* (2)	*Micrococcus lylae*	Species	-	< 1.700	
CICC 23993		*Micrococcus lylae*	Species	-	< 1.700	
CICC 24020	*Moraxella* (1)	*Moraxella osloensis*	Species	-	< 1.700	
CICC 24043	*Paenibacillus* (1)	*Paenibacillus lactis*	Species	-	< 1.700	
CICC 23976	*Staphylococcus* (2)	*Staphylococcus hominis*	Species	-	< 1.700	Housekeeping gene (*tuf*) analysis
CICC 23992		*Staphylococcus warneri*	Species	-	< 1.700	Housekeeping gene (*tuf*) analysis

Table 4. Strains included in the MS database with MALDI-TOF MS scores between 1.7 and 2.0

Strain No.	Genus (Strain number)	16S rRNA gene sequencing		MALDI-TOF MS		Remark
		Species ID	Level of ID	Species ID	Score	
CICC 24011	*Acinetobacter* (1)	*Acinetobacter parvus*	Species	*Acinetobacter junii*	1.822 ± 0.055	
CICC 23960	*Aureimonas* (1)	*Aureimonas altamirensis*	Species	*Aureimonas altamirensis*	1.773 ± 0.037	Physiological and biochemical analysis
CICC 23949	*Bacillus* (5)	*Bacillus cereus*	Species	*Bacillus cereus*	2.024 ± 0.033	Housekeeping gene (*gyrB*) analysis
CICC 23954		*Bacillus infantis*	Species	*Bacillus infantis*	1.900 ± 0.015	
CICC 23959		*Bacillus firmus* or *Bacillus oceanisediminis*	Two different species possible		*Bacillus firmus*	1.850 ± 0.086
CICC 24013		*Bacillus* sp.	Genus	*Bacillus pseudomycoides*	1.818 ± 0.029	Housekeeping gene (*gyrB*) analysis
				Bacillus thuringiensis	1.754 ± 0.030	
				Bacillus cereus	1.784 ± 0.029	
CICC 23950		*Bacillus subtilis* subsp. *subtilis*		Species	*Bacillus subtilis*	2.040 ± 0.110 Housekeeping gene (*gyrA*) analysis
CICC 23948	*Kocuria* (1)	*Kocuria marina*	Species	*Kocuria marina*	1.838 ± 0.062	
CICC 23967	*Micrococcus* (3)	*Micrococcus* sp.	Genus	*Micrococcus luteus*	2.018 ± 0.042	
CICC 24019		*Micrococcus* sp.	Genus	*Micrococcus luteus*	1.764 ± 0.045	
CICC 24033		*Micrococcus* sp.	Genus	*Micrococcus luteus*	1.768 ± 0.039	
CICC 23961	*Moraxella* (5)	*Moraxella osloensis*	Species	*Moraxella* sp.	1.863 ± 0.047	
CICC 24004		*Moraxells osloensis*	Species	*Moraxella osloensis*	1.887 ± 0.115	
CICC 24041		*Moraxella osloensis*	Species	*Moraxella osloensis*	1.796 ± 0.070	
CICC 24044		*Moraxella osloensis*	Species	*Moraxella osloensis*	2.078 ± 0.095	
CICC 24045		*Moraxella osloensis*	Species	*Moraxella osloensis*	1.769 ± 0.030	
CICC 23958	*Paenibacillus* (2)	*Paenibacillus provencensis*	Species	*Paenibacillus* sp.	1.971 ± 0.012	
CICC 24015		*Paenibacillus urinalis*	Species	*Paenibacillus* sp.	1.796 ± 0.058	
CICC 24012	*Pseudomonas* (1)	*Pseudomonas* sp.	Genus	*Pseudomonas nitroreducens*	1.812 ± 0.037	Housekeeping gene (*gyrB*) analysis
				Pseudomonas citronellolis	1.849 ± 0.062	
CICC 23965	*Sphingomonas* (1)	*Sphingomonas paucimobilis*	Species	*Sphingomonas paucimobilis*	2.127 ± 0.147	
CICC 23953	*Staphylococcus* (6)	*Staphylococcus arlettae*	Species	*Staphylococcus arlettae*	1.850 ± 0.154	Housekeeping gene (*tuf*) analysis
CICC 23962		*Staphylococcus epidermidis*	Species	*Staphylococcus epidermidis*	1.973 ± 0.029	Housekeeping gene (*tuf*) analysis
CICC 24059		*Staphylococcus* sp.	Genus	*Staphylococcus cohnii*	1.979 ± 0.122	
CICC 24024		*Staphylococcus* sp.	Genus	*Staphylococcus cohnii*	1.880 ± 0.121	
CICC 24031		*Staphylococcus* sp.	Genus	*Staphylococcus cohnii*	1.908 ± 0.112	
CICC 24036		*Staphylococcus* sp.	Genus	*Staphylococcus cohnii*	1.974 ± 0.185	
CICC 24002	*Stenotrophomonas* (1)	*Stenotrophomonas maltophilia*	Species	*Stenotrophomonas maltophilia*	1.953 ± 0.076	Housekeeping gene (*gyrB*) analysis

Table 5. Strains with no defined species name

Strain No.	Genus (Strain number)	16S rRNA gene sequencing		MALDI-TOF MS		Remark
		Species ID	Level of ID	Species ID Score		
CICC 23999	*Bacillus* (3)	*Bacillus* sp.	Genus	-	< 1.700	Housekeeping gene (*gyrB*) analysis
CICC 23994		*Bacillus megaterium* or *Bacillus aryabhattai* or *Bacillus flexus*	Three different species possible	-	< 1.700	*Bacillus aryabhattai* was not in the MS database
CICC 23989		*Bacillus simplex* or *Bacillus muralis* or *Bacillus butanolivorans*	Three different species possible	-	< 1.700	*Bacillus butanolivorans* was not in the MS database
CICC 23988	*Microbacterium* (1)	*Microbacterium* sp.	Genus	-	< 1.700	
CICC 23982	*Micrococcus* (7)	*Micrococcus* sp.	Genus	-	< 1.700	
CICC 23983		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 23984		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 24000		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 23991		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 24047		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 24055		*Micrococcus* sp.	Genus	-	< 1.700	
CICC 24050	*Pseudomonas* (1)	*Pseudomonas psychfotolerans* or *Pseudomonas oryzihabitans*	Two different species possible	-	< 1.700	*Pseudomonas oryzihabitans* was not in the MS database
CICC 24038	*Stenotrophomonas* (2)	*Stenotrophomonas* sp.	Genus	-	< 1.700	
CICC 24039		*Stenotrophomonas* sp.	Genus	-	< 1.700	

metabolic and psychological divergence between the isolates and those in the MALDI-TOF MS database.

Fourteen strains in Table 6 were identified to genus level by molecular biological methods, but not to species level. The MALDI-TOF MS analysis was unreliable, as the scores were below 1.7. Therefore, other identification tools are needed to characterize these strains in the future.

DISCUSSION

FMCG are strongly associated with lifestyle, and may contain large quantities of water or rich nutrients that promote microbial growth. Formula design must adhere to the regulatory, industrial, or internal requirements of a preservation efficacy test, and a manufactured product must be analyzed for its microbiological content prior to release from the site[32]. However, conventional microbiological detection methods are time consuming as they must allow for microbial growth or incapability of on-site identification, which delays the product release[17]. Therefore, rapid and accurate identification of isolates from products or their manufacturing environment would greatly benefit the FMCG industries.

MALDI-TOF MS is a traditional analytical method that has only recently been considered for rapid microorganism identification[9]. The MALDI-TOF MS equipment has been customized to identify a variety of clinical isolates[9], for example, 92% of 980 routine clinical isolates were correctly identification by MALDI-TOF MS at species-level; on the contrary, conventional identification methods only correctly identified 83.1% of the isolates[26]. The high identification rate is based on the well-developed MS database, such as the Bruker BDAL Biotyper database contains the spectra of bacteria with more than 300 genus and 2200 species. The MS database covers the common bacteria found in industries, such as *Clostridium*, *Escherichia*, *Lactobacillus*, *Paenibacillus* and *Salmonella*[9, 26]. In studies of industrial *Saccharomyces cerevisiae*, MALDI-TOF MS was also confirmed as a rapid and reliable tool for accurate identification[24, 25]. However, the industrial applicability of MALDI-TOF MS is rarely reported. It should be noted that clinical and industrial species are distinct, and the identification accuracy mainly depends on the mass spectra of the database using MALDI-TOF MS. Therefore, if mass spectrometry databases were supplemented with industrially sourced data, the applicability of MALDI-TOF MS would be enhanced, especially in user-customizable databases.

In this work, a total of 152 strains were analyzed with scores lower than 2.0. Two of them, labeled CICC 24037 and CICC 23995, were only identified to the respective family level by 16S rRNA gene sequence analysis, and with MALDI-TOF MS scores below 1.7. The rest 150 strains were classified into 49 genera by the reference method. Forty strains were identified to the genus level by MALDI-TOF MS (Tables 2 and 4), and no misidentification results were found, suggesting the reliability of MALDI-TOF MS. By the reference identification method, 7 genera were not found in the current MS database, including *Asaia*, *Domibacillus*, *Komagataeibacter*, *Luteococcus*, *Oceanobacillus*, *Planomicrobium*, and *Zymomona*, and 58.7% (88/150) strains were not recorded in the MS database. The 88 strains were classified into 42 genera. The results indicated strain divergence of the isolates from FMCG industries to those in the commercial MS database, and the necessity of data deposition for expanding MALDI-TOF MS application.

Very recently, the Bruker MALDI-TOF MS database was updated with additional new species including *Bacillus atrophaeus*, *Bacillus pumilus*, *Kocuria marina*, *Paenibacillus humicus*, and *Pseudomonas oryzihabitans*. This could affect the classification and scores of the strains labeled CICC 23975, CICC 23987, CICC 24027, CICC 24030, and CICC 24050. Every technology has its advantages and limitations. For MALDI-TOF MS, the MS database could affect the application range and identification accuracy. Therefore, database deposition will be continued by researchers to improve the performance of this technology.

The frequencies of strain occurrence are shown in Fig. 1. Some strains were repeatedly identified (more than 5 times), including 29 *Bacillus* strains, 8 *Halomonas* strains, 6 *Lysinibacillus* strains, 12 *Micrococcus* strains, 6 *Moraxella* strains, 8 *Pseudomonas* strains, and 8 *Staphylococcus* strains. *Bacillus* and *Lysinibacillus* can form spores, which are highly tolerant to extreme environmental stress[16,18].

Halomonas can survive over a wide range of salinity, and is resistant to surface active agents[8, 10]. These genera demand attention, as they are often isolated in cosmetics[12, 20, 23]. Some *Pseudomonas*, *Moraxella* and *Staphylococcus* species are implicated in human diseases, and should not be present in consumer products[3, 21, 30].

In conclusion, 152 bacterial strains were analyzed by MALDI-TOF MS with scores lower than 2.0, and supplemented with 16S rRNA and housekeeping genes sequence analysis and (where necessary) physiological and biochemical analyses. By depositing the generated mass spectrometry data in the MS database, we could tailor the use of MALDI-TOF MS to FMCG industrial microbial detection, greatly expanding the applicability of the method. Moreover, rapid and accurate identification of bacterial isolates from various samples enables agile, actionable mitigation planning, ensuring product quality and consumer safety. Expanding the database to include a broader set of industrially-source microbes will increase the success rate of identifications.

We note that the system did not misidentify or return a nonidentifiable identification for any frank human pathogens. This supports the validity of the Biotyper system for the identification of bacteria that could potentially pose a human health risk, which is critical for use of the system in both manufacturing and health care environments. This is in agreement with the recent approval for use by US Food and Drug Association via a 510(k) clearance in 2013, and expanded upon with a second 510(k) clearance in 2015, and clearance by the China Food and Drug Association in 2014, for use of the Bruker IVD database for in vitro diagnostics.

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