Development of a rapid identification method for food bacteria and molds : MALDI-TOF MS Project

ILSI Japan, Microbiological Food Safety Task Force
NITE Biological Resource Center (NBRC)

Mitsuru Katase

COI Disclosure Information
Mitsuru Katase

- *I have no financial relationships to disclose.*

Disclaimer

- *The opinions expressed are those of the presenter and do not reflect those of the International Life Sciences Institute.*

Important emerging issues of food safety in Japan

- Increase in microbiological risk in whole food chain due to global warming
- Increase in expectation that functional foods will extend healthy life-span
- Creation of foods with novel and/or high functionalities generated by progress in food science
- Pursuit of higher food safety and reliability in Japanese Society: “ZERO risk” belief
- Expansion of a strong request of animal testing ban from NGO to a food industry

Scientific challenges to address the issues

Further progressing in Risk assessment technologies

- Deepening precise technologies for microbiological risk assessment
  - identification of microbes
  - flora analysis
- Development of new strategies and technologies for assuring safety of novel functional foods
- Development of non-animal testing methodologies for safety evaluation of foods

Promoting researches for supporting risk communication

- Risk recognition
- Two-way risk communication methods

Food Safety Research Committee
- Microbiological Food Safety Task Force

Task Force Objectives

The task force aims to gather and research scientific information on food microorganisms (especially harmful microbes) that is useful to the food products industry, from a broad perspective. It will also disseminate the results to all the food safety's stakeholders through **publications**, **symposiums**, and **lectures**, under the name of ILSI Japan.

Activities of Microbiological Food Safety Task Force

1. Development of a rapid identification method for food bacteria and molds : MALDI-TOF MS project
2. Harmonization of inspection methods of spore forming bacteria
3. Information gathering of microbiological risk of chilled distribution foods
4. Reduction of consumer complaints of PET bottle beverages after opening
5. NGS Project : Collaboration with ILSI Europe

MALDI-TOF MS Project

Background:

- Spoilage of food products due to microorganisms is one of major problems.
- Information on the safety risk should be obtained as soon as possible when the spoilage occurs.
- Identification of the spoilage microbes is extremely essential.
- Current methods for mold identification are very time-consuming (hours-days).

Goal:

- To develop a novel rapid technique for identification of food bacteria and molds and verify the standard protocol.

MALDI-TOF MS Project
Microbe Identification using MALDI-TOF MS

- What is MALDI-TOF MS?

  Matrix-Assisted Laser Desorption Ionization-
  Time of Flight Mass Spectrometry

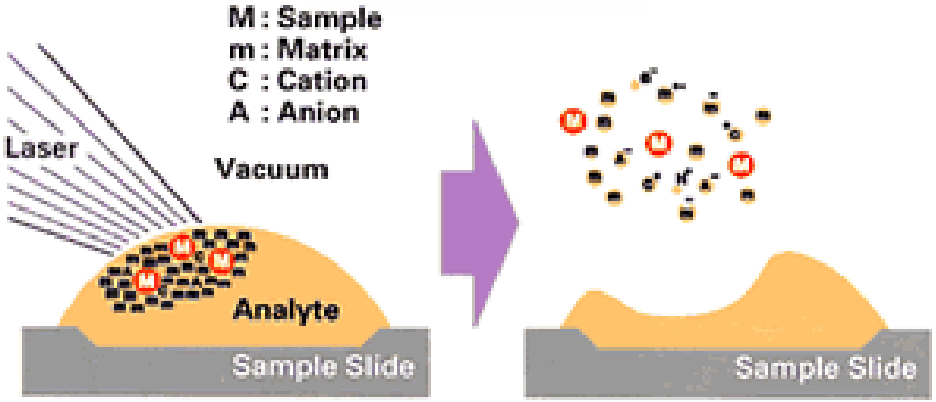
- A fingerprint (mass spectral pattern) of molecules composed mainly of microorganism-specific ribosomal protein is obtained by MS, followed by searching /matching with a known microbial strain library and identifying the target bacterial species.

- Expected major advantages by applying the technology to microbe identification;

"Identification will be drastically quick."

Principle of MALDI-TOF MS

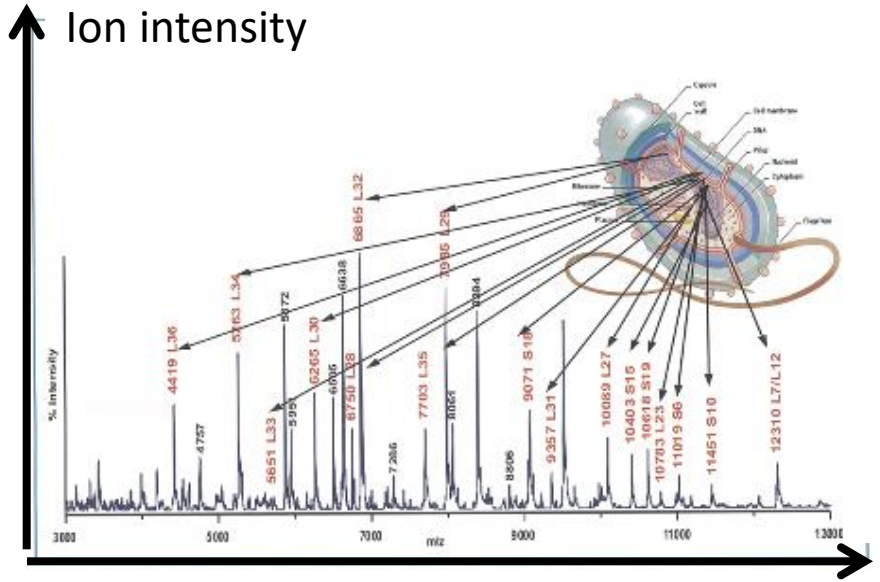
Ionization



Before laser irradiation **After laser irradiation**

Aanalytes are mixed with biopolymer (a matrix) and are Ionized by strong laser irradiation.

Mass spectrum

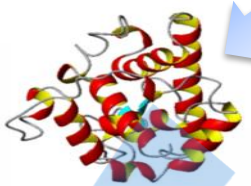


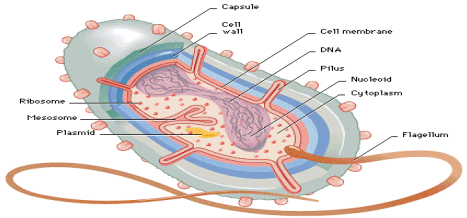
m/z
(Mass-to-charge ratio)

The ions generated by the irradiation are analyzed by mass spectrometry based on time of flight of the ions.

microorganisms

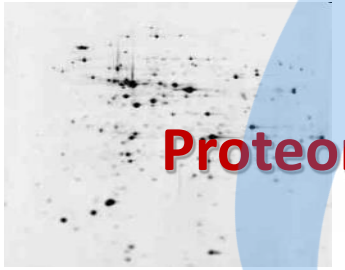
protein





DNA

mRNA



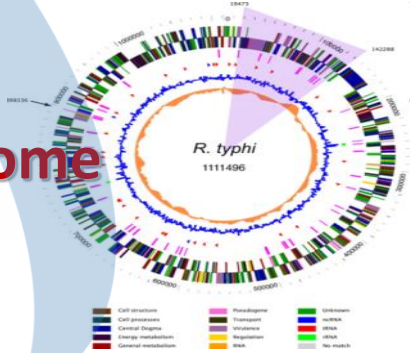
Proteome

Identification

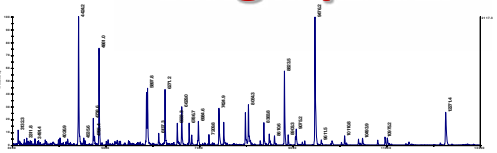
In minutes

low cost: $0.20/sample

genome

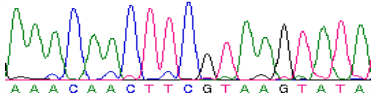


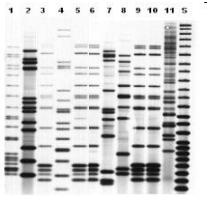
Mass Fingerprint



MALDI-TOF MS

DNA Fingerprint





DNA sequencer ⇒hours

MALDI-TOF MS Project
Microbe strain library

- Microbial strain library is very important for a fingerprinting with molecules composed mainly of microorganism-specific ribosomal protein.

- But, as this technique started in the clinical field, the library included mainly clinical bacteria.

MALDI-TOF MS Project
Microbe strain library

- Nobody had tried to expand the library for food industry.
- Microbiological Food Safety Task Force as one made much effort to convince the equipment manufacturers to expand the library for spoilage bacteria in food industry and provided them with known microbial strains owned by Japan Canners Association.

Examples of the list of food/beverage spoilage microbes

Yeast

classification	genus	species	thermophilic / chemical torelans /and so on	detection frequency /fermentation	must	notes
Yeast	Saccharomyces	cerevisiae	○	○	●	DSMZ(Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.)or CBS(Centraal bureau voor Schimmelcultures)have many collections.
		bayanus		○	●	
		pastorianus		○	●	
		capsularia				
	Saccharomycodes	ludwigii				
		sinensis				
	Shizosaccharomyces	pombe				
		cryophilus				
		japonicus				
	Zygosaccharomyc	bailii	○		●	
		bisporus	○		●	
		rouxii	○		●	
	Candida	albicans		○	●	
		famata		○	●	
		krusei		○	●	
		guilliermondii		○	●	
		parapsilosis		○	●	
	Brettanomyces	anomalus	○		●	
		bruxellensis	○		●	
		naardenensis	○		●	
		custersianus	○		●	
		nanus	○		●	

Species with high attentions are highlighted with symbols (●).

Examples of the list of food/beverage spoilage microbes

Molds

classification	genus	species	mycotoxin / thermophilic / chemical torelans	detection frequency	must	notes
Mold	Aspergillus	fumigatus	○	○	●	DSMZ(Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.)or CBS(Centraal bureau voor Schimmelcultures) have many collections.
		ochraceus	○	○	●	
		versicolor	○	○	●	
		parasiticus	○	○	●	
		flavus	○		●	
		nomius	○		●	
		nidulans		○	●	
		niger		○	●	
		oryzae		○	●	
		sojae		○	●	
		terreus		○	●	
		sydowii		○	●	
		amstelodami		○	●	
		unguis		○	●	
		clavatus		○	●	
		terreus		○	●	
		candidus		○	●	
		clavatus		○	●	
		fischeri		○	●	
		penicilloides		○	●	
		restrictus		○	●	
		sclerotiorum				
		tamarii				
		equitus				
		glaucus				
		ustus				
		yaponicus				

Examples of the list of food/beverage spoilage microbes

Bacillus

classification	genus	species	thermophilic / chemical torelans/acidophilic/pathogenicity	detection frequency	must	notes
spore-form bacteia	Bacillus	acidiceler				Japan Canners Association
		acidicola	○		●	
		aerophilus				
		altitudinis				
		amyloliquefaciens		○	●	
		anthracis	○		●	
		aquimaris				
		arsenicus				
		atrophaeus		○	●	
		baekryungensis				
		barbaricus				
		bataviensis				
		boroniphilus				
		butanolivorans				
		cereus		○	●	
		circulans	○	○	●	
		coagulans	○		●	
		cohnii				
		firmus		○	●	
		flexus		○	●	
		foraminis				
		fumarioli		○	●	
		funiculus				
		gelatini				
		ginsengihumi		○	●	
		halodurans				
		horneckiae				
		lehensis				
		lentus				

Examples of the list of food/beverage spoilage microbes

Alicyclobacillus (TAB)

classification	genus	species	off-flavour produce	detection frequency	must	notes
TAB (Thermo acidophilic Bacilli)	Alicyclobacillus	acidoterrestris	+	○	●	ISBT(International Society of Beverage Technologists) have many collections.
		acidocaldarius	−	○	●	
		sp.(genomic sp.1)	−	○	●	
		herbarius	+		●	
		acidiphilus	+		●	
		hesperidum subsp. aigle	+		●	
		hesperidum	−		●	
		cycloheptanicus	−			
		pomorum	−			
		contaminans	−			
		disulfidooxidans	−			
		fastidiosus	−			
		kakegawensis	−			
		macrosporangiidus	−			
		sacchari	−			
		sendaiensis	−			
		shizuokensis	−			
		tolerans	−			
		vulcanalis	−			

The list of food/beverage spoilage microbes

- We provided the equipment manufacturers with the list of various food/beverage spoilage microbes needed in food industry.
  But, it is time-consuming to expand the library in their efforts.

- Therefore, we decided to move forward to the more practical collaboration with JCA.

Introduction of the JCA (Japan Canners Association) collection of spore-forming bacteria

- JCA has an in-house culture collection of wild spoilage strains from varieties of canned and retort foods in their history. It amounts to 340 strains in total, including 178 strains of aerobic spore-formers (such as Bacillus), 94 strains of anaerobic spore-formers (such as Clostridium), and 68 strains of thermophilic anaerobic spore-formers.

Introduction of the JCA (Japan Canners Association) collection of spore-forming bacteria

- Since their collection are mainly characterized physiologically in the past, they agreed to share the strains with ILSI Japan and MALDI-TOF MS manufacturers for the purpose of sequencing and MALDI-TOF MS database establishment.
- 16SrDNA sequencing was carried out on the entire collection of JCA. Identification was based on the upper 500 bp of 16SrDNA, while the full sequence was applied if the result was not decisive according to the International Clinical and Laboratory Standards.

Re-identification of JCA culture collection by 16SrDNA sequencing. A)Aerobic spore-formers, B)Thermophilic aerobic spore-formers C)Anaerobic spore-formers, D)Thermophilic anaerobic spore-formers

Originally identified by physiological characterization in JCA		Results of reidentification	
A) Aerobic spore-formers			strains
Paenibacillus polymyxa	6	P. polymyxa, P.poeriae	4
		P. terrae	2
Paenibacillus macerans	7	P. macerans, P.thermophilus	6
		Paenibacillus chibensis	1
B) Thermophilic aerobic spore-formers			
Geobacillus stearothermophilus	22	G. stearothermophilus	18
		Bacillus smithii	4
Bacillus sporothermodurans	8	B.sporothermodurans	8
B. coagulans	25	B. coagulans	25
C) Anaerobic spore-formers			
Clostridium sporogenes	67	C.sporogenes	67
Clostridium bifermantans	4	C.bifermantans	4
Clostridium butyricum	6	C.butyricum	2
		C.beijerinkii	2
		C.acidisoli	2
Clostridium pasteurinum	17	C.pasteurinum	13
		C.arbusti	3
		C.tyrobutyricum	1
D) Thermophilic anaerobic spore-formers			
Desulfotomaculum nigrificans	6	D.nigirificans or D.carboxydivorans	6
Moorella thermoacetica	10	M.thermoacetica	10
Thermoanaerbacterium thermosaccaharolyticum	35	T.thermosaccaharolyticum	20
		Thermoanaerbacterium sp(T. aoteoroens)	13
		Thermoanaerbacterium sp(T. xylanolyticum)	2
Thermoanaerbacterium thermohydrosulfuricum	17	T.mathranii	17
		T.thermohydrosulfuricus	
		Caldanaerobius polysaccahlorityicum/zeae	

Full 16SrDNA sequencing results of strains of JCA culture collection were registered to NCBI gene bank.

Strain No.	Species name
JCA-1201	Bacillus licheniformis
JCA-1313	Paenibacillus favisporus
JCA-1422	Bacillus licheniformis
JCA-1602	Bacillus safensis
JCA-1805	Paenibacillus terrae
JCA-1904	Paenibacillus thermophilus
JCA-1906	Paenibacillus thermophilus
JCA-5511	Clostridium pasteurianum
JCA-5602	Thermoanaerobacterium aotearoense
JCA-5603	Thermoanaerobacterium thermosaccharolyticum
JCA-5637	Thermoanaerobacterium thermosaccharolyticum
JCA-5801	Moorella thermoacetica
JCA-5901	Thermoanaerobacterium mathranii
JCA-5920	Caldanaerobius sp.
IAM1227	Paenibacillus thermophilus
IAM1243	Paenibacillus thermophilus
DSM574	Desulfotomaculum nigrificans
DSM14880	Desulfotomaculum carboxydivorans

Expecting number of new strains and species in updated MALDI-TOF MS database of two manufacturers

A) Bruker Daltonics Inc.

Genus	species	The number of strain
Bacillus	altitudinis	2
Paenibacillus	barengoltzii	1
Geobacillus	thermoglucosidasius	3
Alicyclobacillus	cycloheptanicus	3
Zygosaccharomyces	bisporus	1
	rouxii	2
total	6	12

B) BioMerieux

Genus	species	The number of strain
Bacillus	safensis	2
Bacillus	lentus	1
Bacillus	smithii	4
Bacillus	thermoamylovorans	1
Paenibacillus	macerans	2
Paenibacillus	polyyxa	3
Paenibacillus	favisporus	4
Paenibacillus	phoenicis	1
Paenibacillus	terrae	2
Virgibacillus	proomii	8
Clostridium	sporogenes	8
Clostridium	sp.	2
Clostridium	acidisoli	2
Clostridium	arbusti	2
Clostridium	pasteurianum	12
Clostridium	tyrobutyricum	1
Thermoanaerobacterium	thermosaccharolyticun	6
Thermoanaerobacterium	mathranii	3
Thermoanaerobacterium	aciditorelans	2
Thermoanaerobacterium	aotearoense	11
Cladanaerobius	sp.	2
Desulfotomaculum	nigrificans	2
total	22	81

Increase in the data base of strains/species causing food spoilage results in increase of agreement rate of identification.

MALDI-TOF MS Project

Key to success

- The key to success so far was to design an elaborate framework of the technique and to network various stakeholders.

Project members

- Fourteen companies in food industry
- More than 20 expert members

MALDI-TOF MS Project

Current:

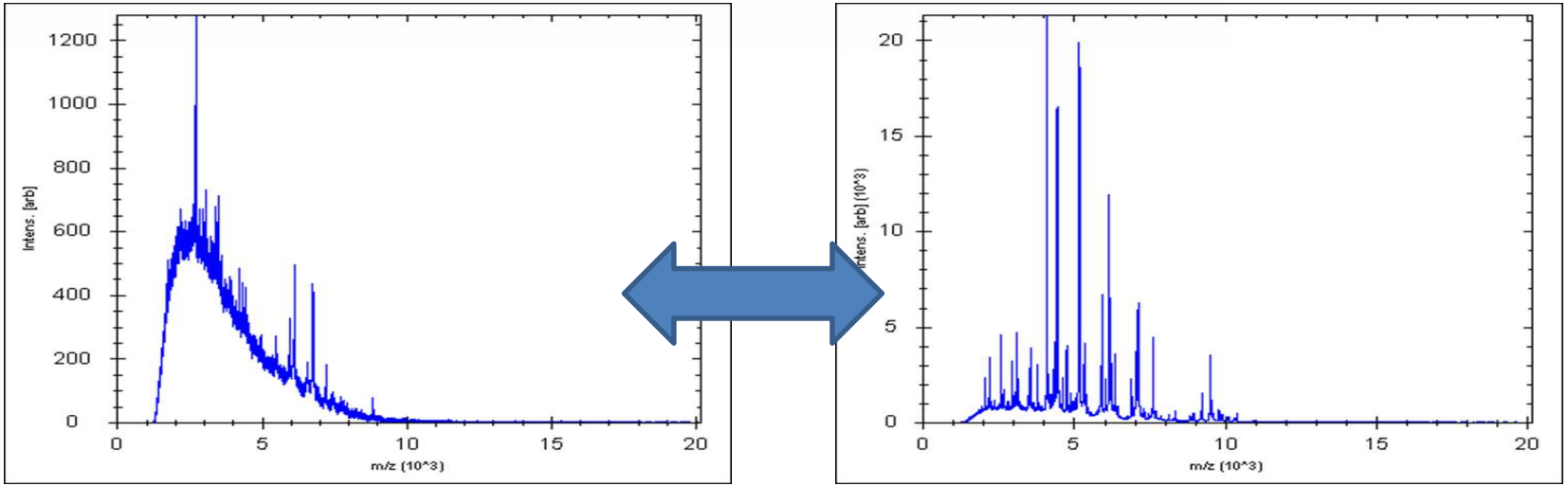
Finished a feasibility study of application of new methodologies to identification of food microbes. However, mold identification by MALDI-TOF MS is still unstable and difficult.

Has started the collaborative investigation of the methodologies with NITE Biological Resource Center (NBRC)* .

* *A national research institute of Japan*

Mold identification

- Different spectra from same colony



- Different growth rate from different species

Stable identification by MALDI-TOF MS

Bacteria	Excellent ～ Fair
Yeast	Very good
Mold	Fair ～ Poor

Investigating improved methodologies using some typical food molds for rapid identification.

MALDI-TOF MS Project

Expected outcome - impact on health:

- MALDI-TOF MS technique will drastically speed up the microbe identification to ensure the microbiological food safety of products and to solve microbiological problems quickly.
- Increase of agreement rate of identification for spoilage bacteria results in the same above.
- All stakeholders in the food industry will share the information on the new technique with each other, which will surely strengthen the whole industry and benefit the consumers.

Thank you for your attention.