MALDI Biotyper

- Fast & Accurate Identification of Microorganisms

BRUKER

MALDI Biotyper: an alternative to identify microorganisms

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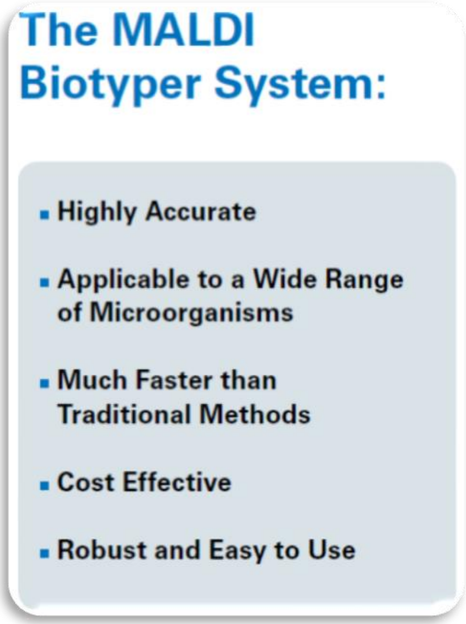
11 Nov 2013

Introduction

- Traditional methods of identifying microorganisms
  - Gram stain
  - Culture on selective media
  - Biochemical tests
  - 16S sequencing
- Some limitations of traditional methods
  - Time consuming, labor-intensive
  - Expensive test media and reagents

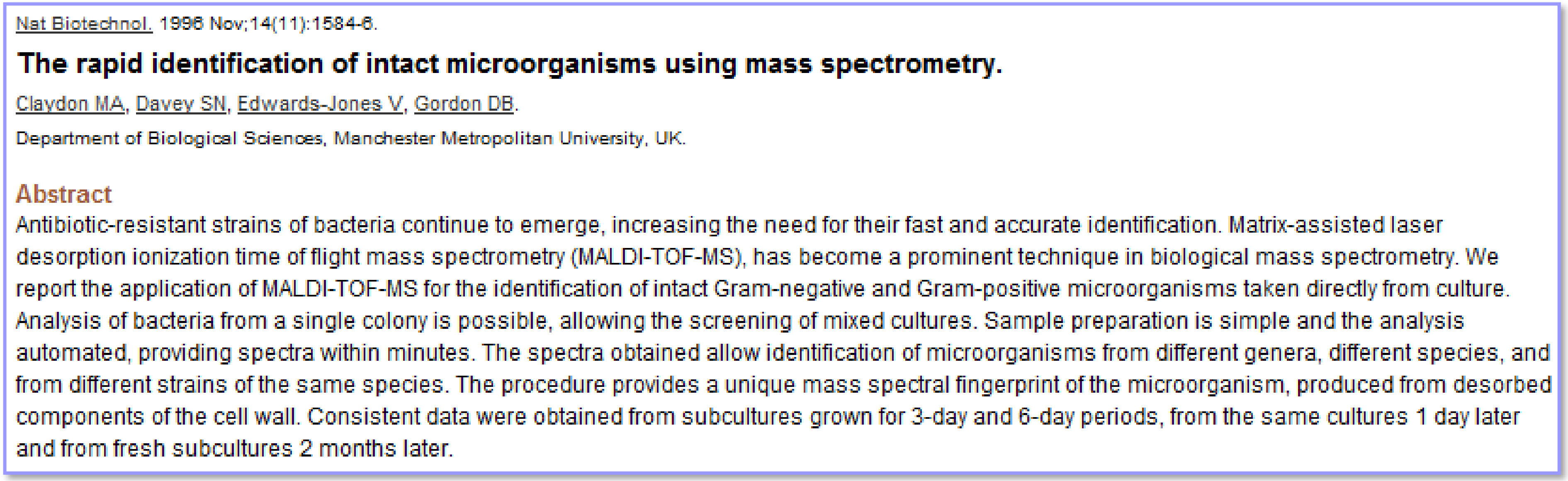


The MALDI Biotyper System:

- Highly Accurate
- Applicable to a Wide Range of Microorganisms
- Much Faster than Traditional Methods
- Cost Effective
- Robust and Easy to Use

Introduction

1996: identification of Gram (-) and Gram (+) bacteria taken directly from culture using MALDI-TOF MS.



Nat Biotechnol. 1996 Nov;14(11):1584-6.

The rapid identification of intact microorganisms using mass spectrometry.

Claydon MA, Davey SN, Edwards-Jones V, Gordon DB.

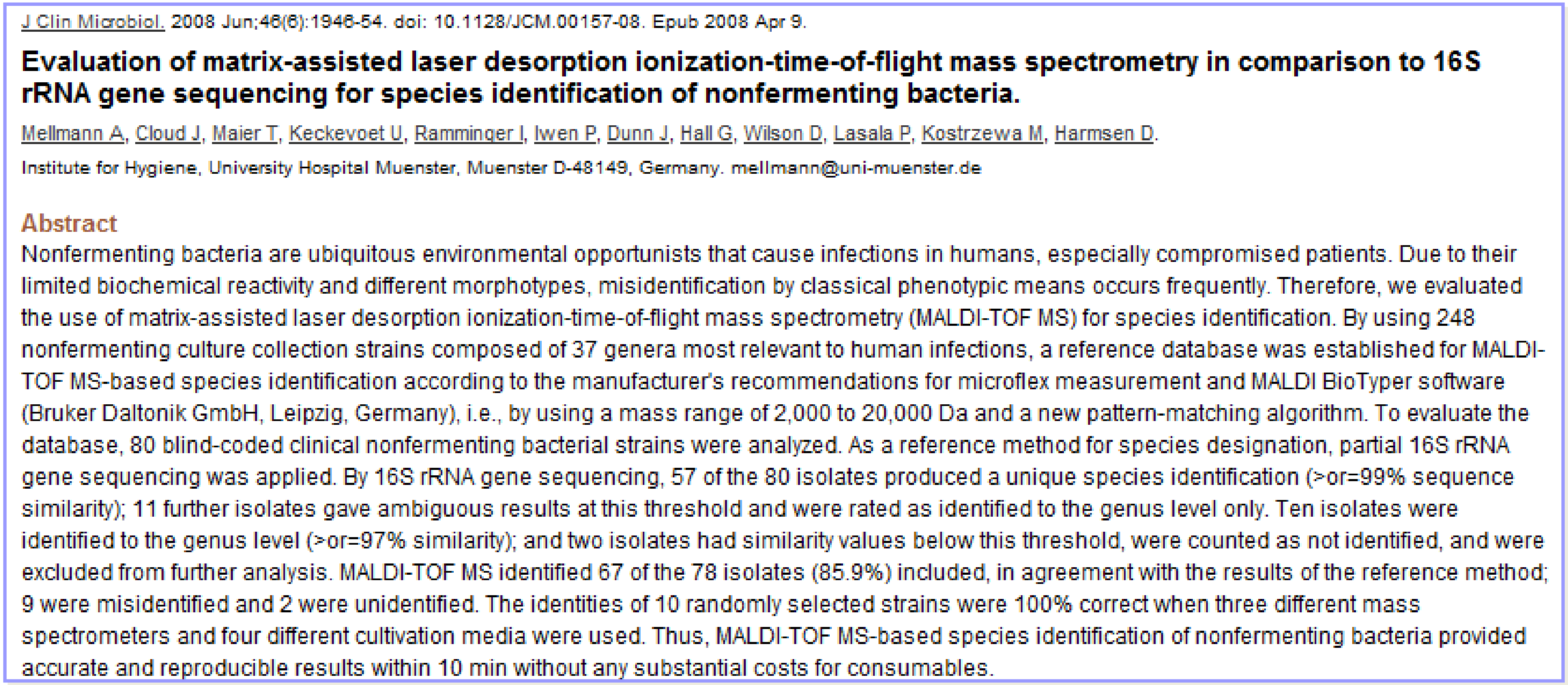
Department of Biological Sciences, Manchester Metropolitan University, UK.

Abstract

Antibiotic-resistant strains of bacteria continue to emerge, increasing the need for their fast and accurate identification. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS), has become a prominent technique in biological mass spectrometry. We report the application of MALDI-TOF-MS for the identification of intact Gram-negative and Gram-positive microorganisms taken directly from culture. Analysis of bacteria from a single colony is possible, allowing the screening of mixed cultures. Sample preparation is simple and the analysis automated, providing spectra within minutes. The spectra obtained allow identification of microorganisms from different genera, different species, and from different strains of the same species. The procedure provides a unique mass spectral fingerprint of the microorganism, produced from desorbed components of the cell wall. Consistent data were obtained from subcultures grown for 3-day and 6-day periods, from the same cultures 1 day later and from fresh subcultures 2 months later.

Introduction

2008: first publication using Biotyper strategy (Bruker).



J Clin Microbiol. 2008 Jun;46(6):1946-54. doi: 10.1128/JCM.00157-08. Epub 2008 Apr 9.

Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria.

Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, Iwen P, Dunn J, Hall G, Wilson D, Lasala P, Kostrzewa M, Harmsen D.

Institute for Hygiene, University Hospital Muenster, Muenster D-48149, Germany. mellmann@uni-muenster.de

Abstract

Nonfermenting bacteria are ubiquitous environmental opportunists that cause infections in humans, especially compromised patients. Due to their limited biochemical reactivity and different morphotypes, misidentification by classical phenotypic means occurs frequently. Therefore, we evaluated the use of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) for species identification. By using 248 nonfermenting culture collection strains composed of 37 genera most relevant to human infections, a reference database was established for MALDI-TOF MS-based species identification according to the manufacturer's recommendations for microflex measurement and MALDI BioTyper software (Bruker Daltonik GmbH, Leipzig, Germany), i.e., by using a mass range of 2,000 to 20,000 Da and a new pattern-matching algorithm. To evaluate the database, 80 blind-coded clinical nonfermenting bacterial strains were analyzed. As a reference method for species designation, partial 16S rRNA gene sequencing was applied. By 16S rRNA gene sequencing, 57 of the 80 isolates produced a unique species identification (>or=99% sequence similarity); 11 further isolates gave ambiguous results at this threshold and were rated as identified to the genus level only. Ten isolates were identified to the genus level (>or=97% similarity); and two isolates had similarity values below this threshold, were counted as not identified, and were excluded from further analysis. MALDI-TOF MS identified 67 of the 78 isolates (85.9%) included, in agreement with the results of the reference method; 9 were misidentified and 2 were unidentified. The identities of 10 randomly selected strains were 100% correct when three different mass spectrometers and four different cultivation media were used. Thus, MALDI-TOF MS-based species identification of nonfermenting bacteria provided accurate and reproducible results within 10 min without any substantial costs for consumables.

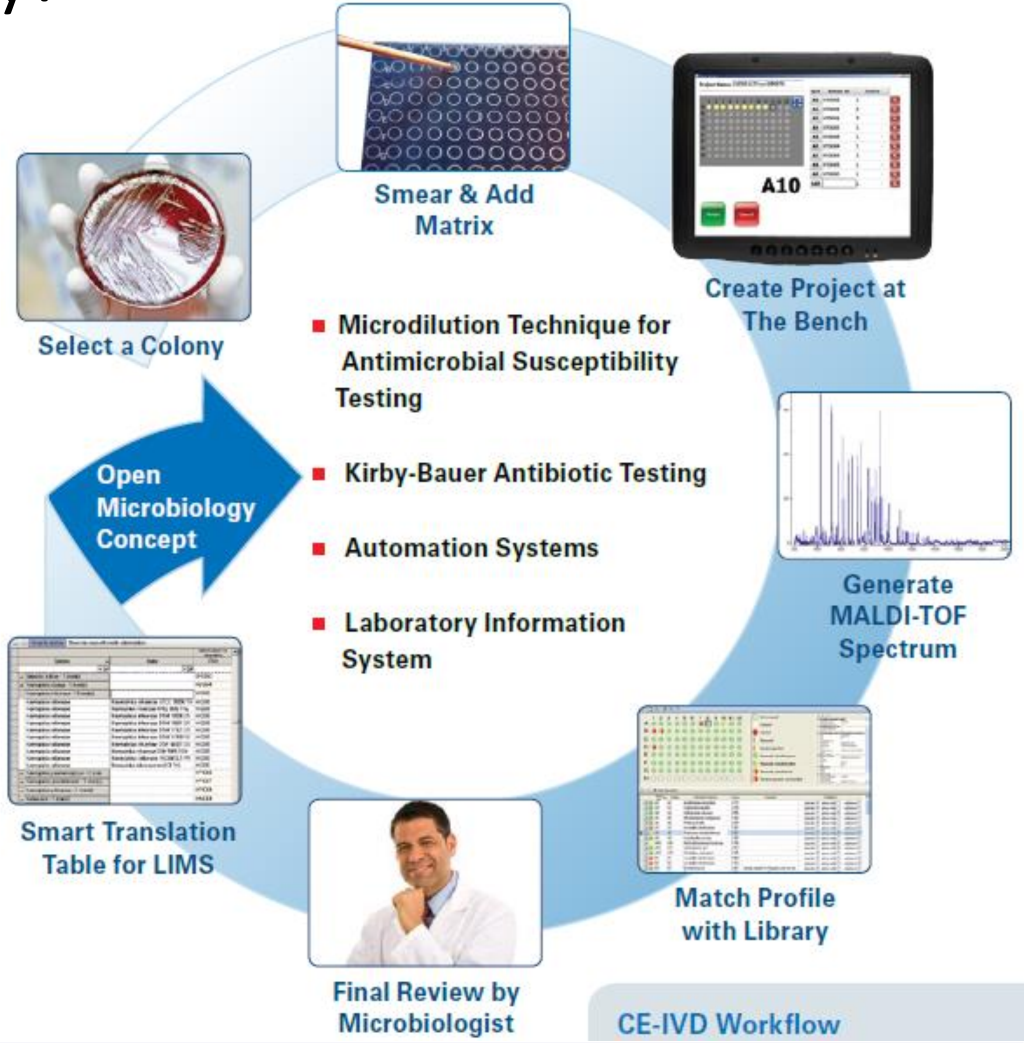
Introduction

What is the Biotyper strategy?

- Identifies microorganisms using MALDI-TOF MS to measure a unique molecular fingerprint of an organism.

- Measures highly abundant proteins that are found in all microorganisms.

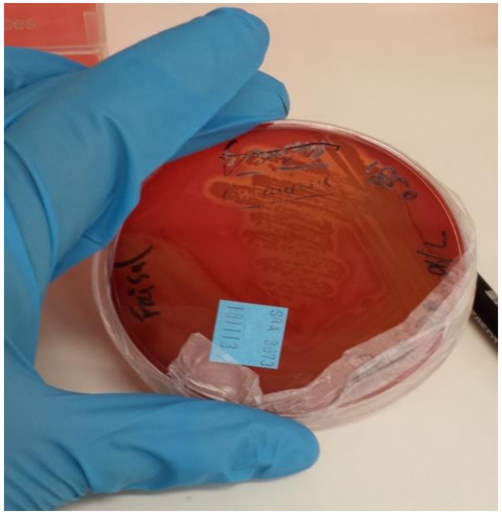
- Characteristic patterns of these highly abundant proteins are used to reliably and accurately identify a particular microorganism down to the species level by matching the respective pattern with an extensive open database.



- Microdilution Technique for Antimicrobial Susceptibility Testing
- Kirby-Bauer Antibiotic Testing
- Automation Systems
- Laboratory Information System

Sample preparation

Culture stored at RT or 4°C



Method 1a:

direct transfer of one CFU

Dead easy! The matrix kills the bacteria.

For non pathogenic bacteria (the goodies)

Method 1b:

Method 1 + 1uL FA

In case method 1 fails...

Method 2:

EtOH/FA extraction

FA lyses cell wall and releases proteins.

For non sporulating bacteria (the badies)

Method 3:

TFA extraction

TFA, stronger acid than FA, breaks off the spores.

For sporulating bacteria (the nasties!)

NB: method 2 is claimed to be the most efficient. If safe lab practices are observed, method 1 can be used for any type of bacteria.

Sample spotting

Spot CFU or supernatant
onto target plate.

↓

Air dry.

↓

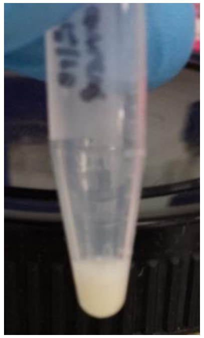
cover with 2 µL HCCA matrix solution.

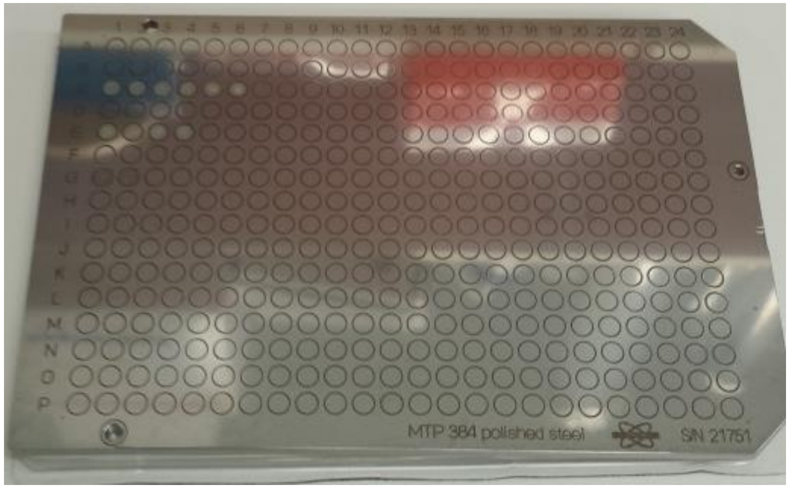
↓

Air dry.

↓

MALDI-TOF analysis



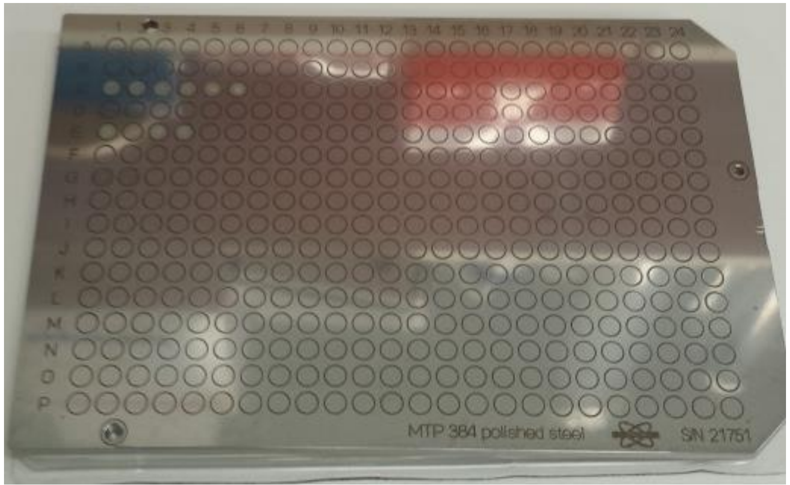


MTP 384 polished steel target plate

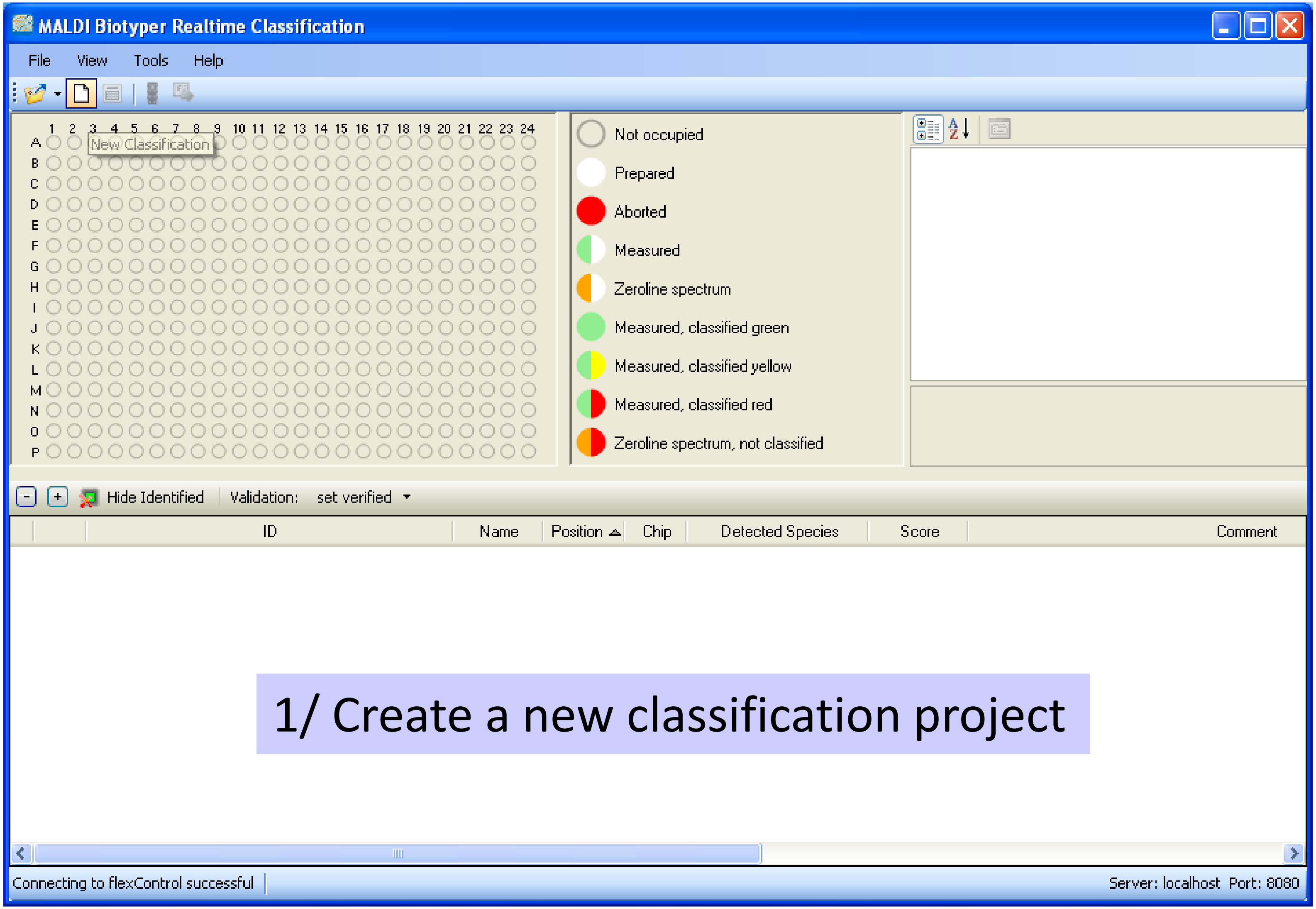
HCCA: α-cyano-4-hydroxycinnamic acid

MALDI-TOF analysis

UltrafleXtreme MALDI-TOF/TOF MS (Bruker)
- 10 µm laser diameter for higher resolution
- 1 kHz speed



Biotyper workflow



- ○ Not occupied
- ○ Prepared
- ● Aborted
- ◐ Measured
- ◐ Zeroline spectrum
- ● Measured, classified green
- ◐ Measured, classified yellow
- ◐ Measured, classified red
- ◐ Zeroline spectrum, not classified

Validation: set verified

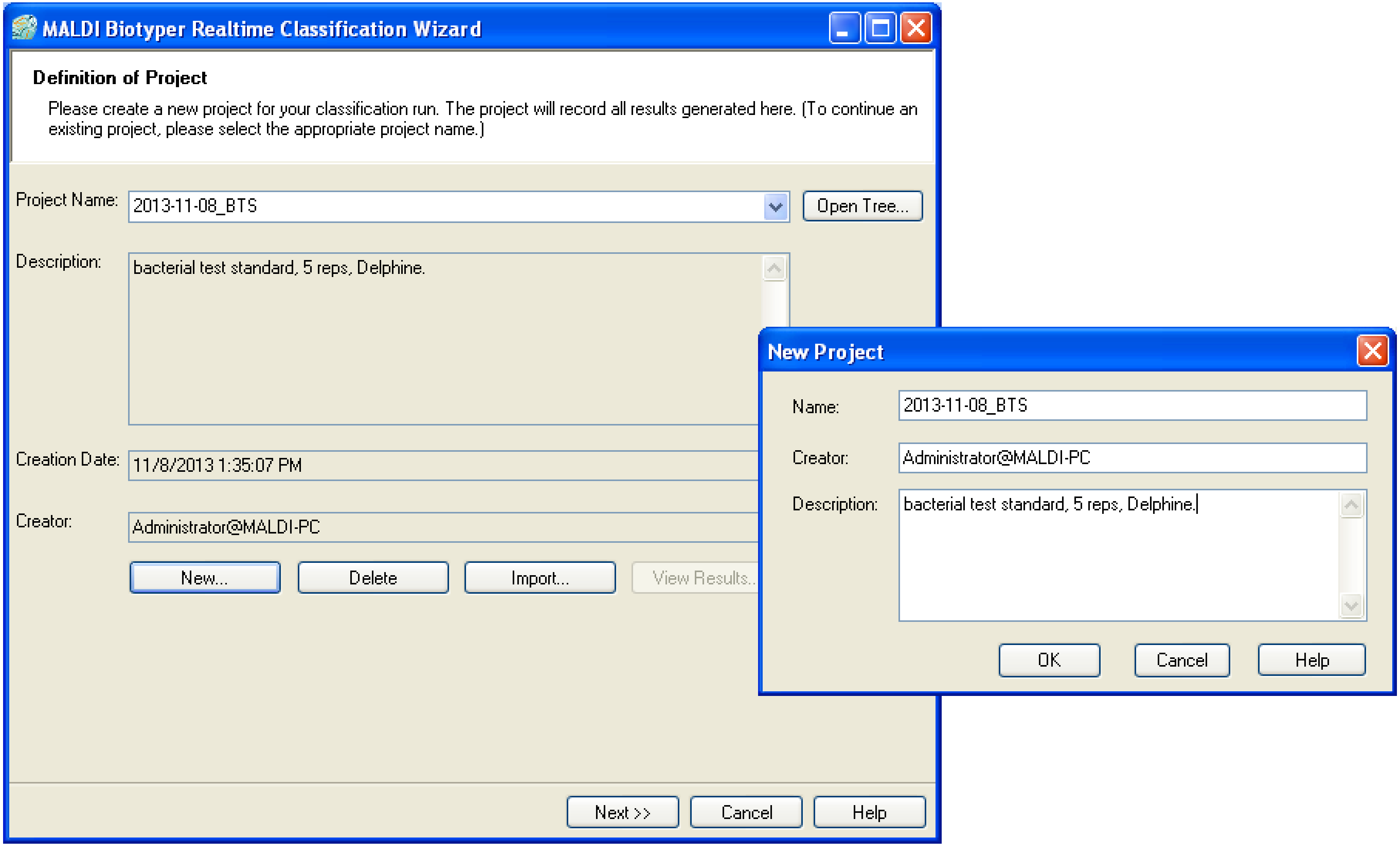
ID	Name	Position ▲	Chip	Detected Species	Score	Comment

Connecting to flexControl successful

Server: localhost Port: 8080

1/ Create a new classification project

Biotyper workflow



MALDI Biotyper Realtime Classification Wizard

Definition of Project

Please create a new project for your classification run. The project will record all results generated here. (To continue an existing project, please select the appropriate project name.)

Project Name: 2013-11-08_BTS

Description: bacterial test standard, 5 reps, Delphine.

Creation Date: 11/8/2013 1:35:07 PM

Creator: Administrator@MALDI-PC

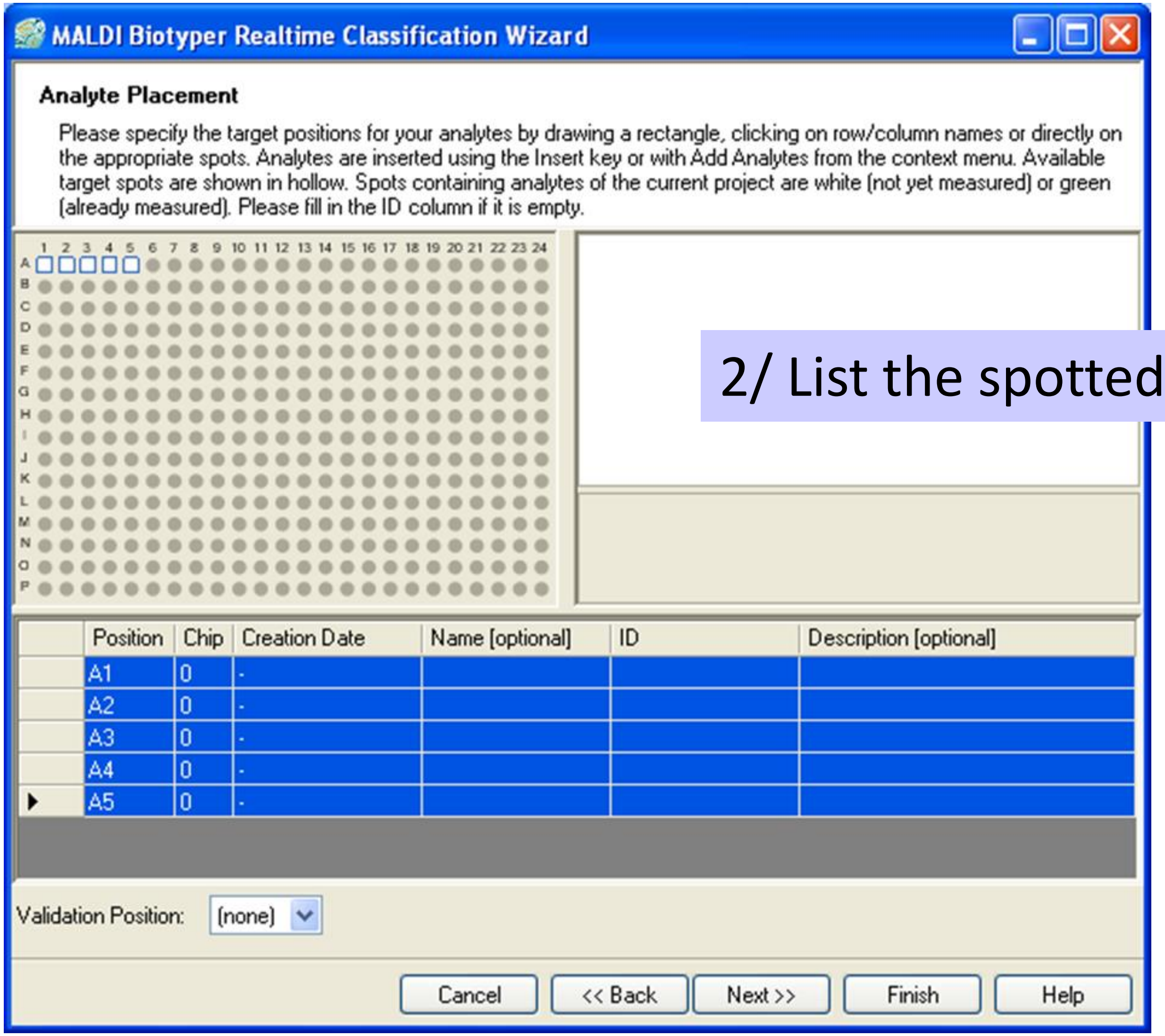
New Project

Name: 2013-11-08_BTS

Creator: Administrator@MALDI-PC

Description: bacterial test standard, 5 reps, Delphine.

Biotyper workflow



Analyte Placement

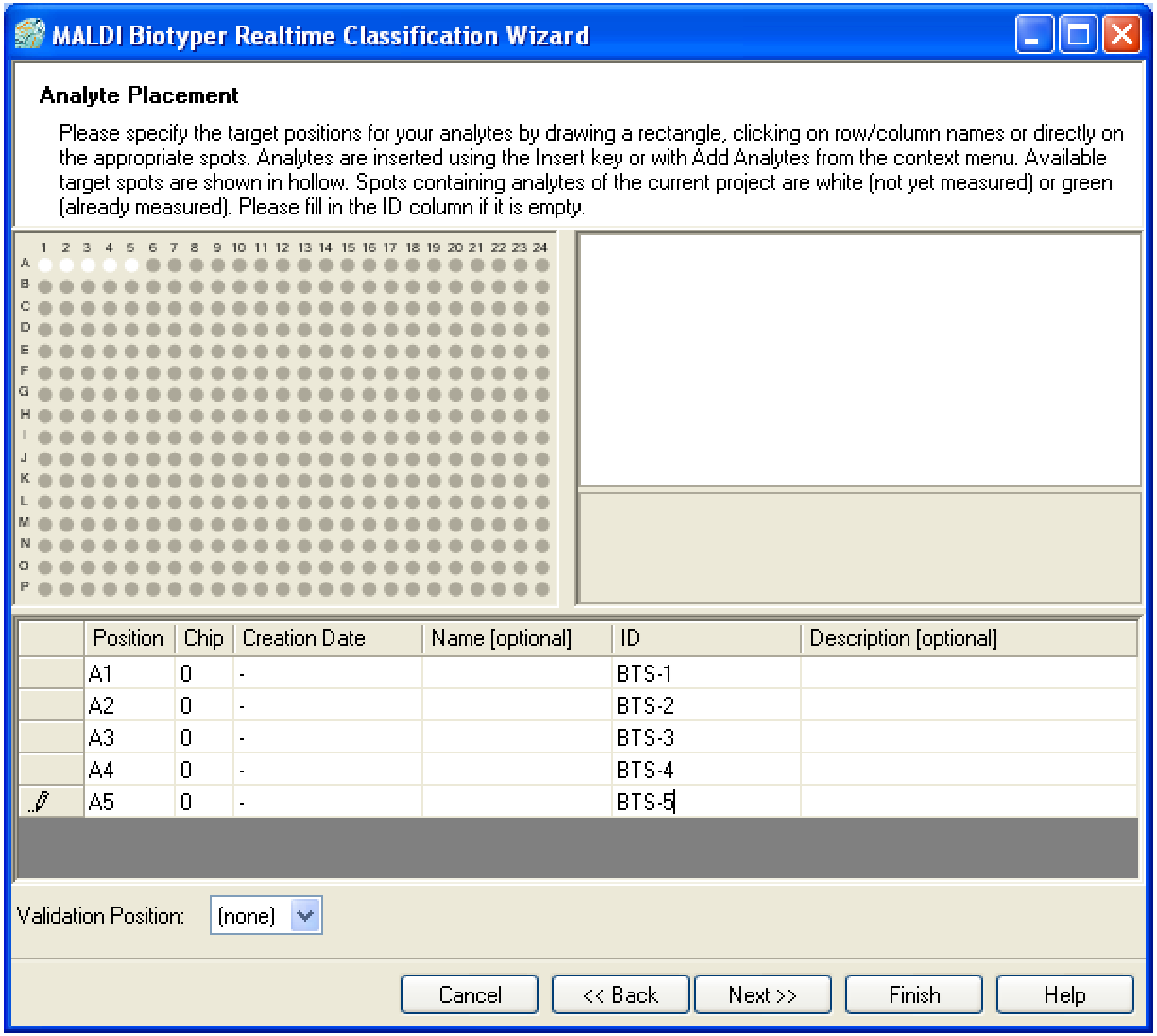
Please specify the target positions for your analytes by drawing a rectangle, clicking on row/column names or directly on the appropriate spots. Analytes are inserted using the Insert key or with Add Analytes from the context menu. Available target spots are shown in hollow. Spots containing analytes of the current project are white (not yet measured) or green (already measured). Please fill in the ID column if it is empty.

	Position	Chip	Creation Date	Name [optional]	ID	Description [optional]
	A1	0	-			
	A2	0	-			
	A3	0	-			
	A4	0	-			
▶	A5	0	-			

Validation Position: (none)

2/ List the spotted analytes

Biotyper workflow



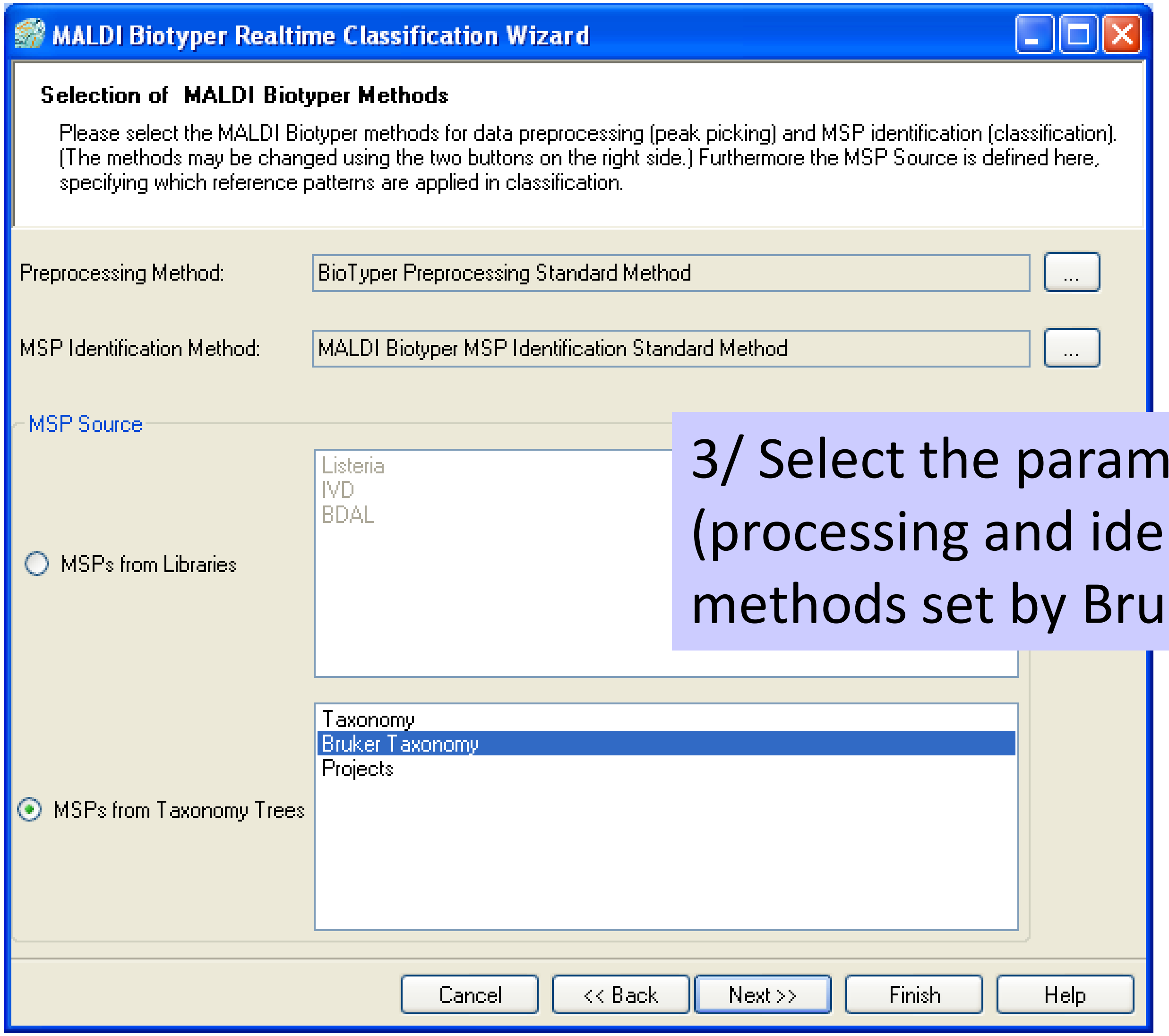
Analyte Placement

Please specify the target positions for your analytes by drawing a rectangle, clicking on row/column names or directly on the appropriate spots. Analytes are inserted using the Insert key or with Add Analytes from the context menu. Available target spots are shown in hollow. Spots containing analytes of the current project are white (not yet measured) or green (already measured). Please fill in the ID column if it is empty.

	Position	Chip	Creation Date	Name (optional)	ID	Description (optional)
	A1	0	-		BTS-1	
	A2	0	-		BTS-2	
	A3	0	-		BTS-3	
	A4	0	-		BTS-4	
	A5	0	-		BTS-5	

Validation Position: (none)

Biotyper workflow



Selection of MALDI Biotyper Methods

Please select the MALDI Biotyper methods for data preprocessing (peak picking) and MSP identification (classification). (The methods may be changed using the two buttons on the right side.) Furthermore the MSP Source is defined here, specifying which reference patterns are applied in classification.

Preprocessing Method: BioTyper Preprocessing Standard Method

MSP Identification Method: MALDI Biotyper MSP Identification Standard Method

MSP Source

○ MSPs from Libraries

Listeria
IVD
BDAL

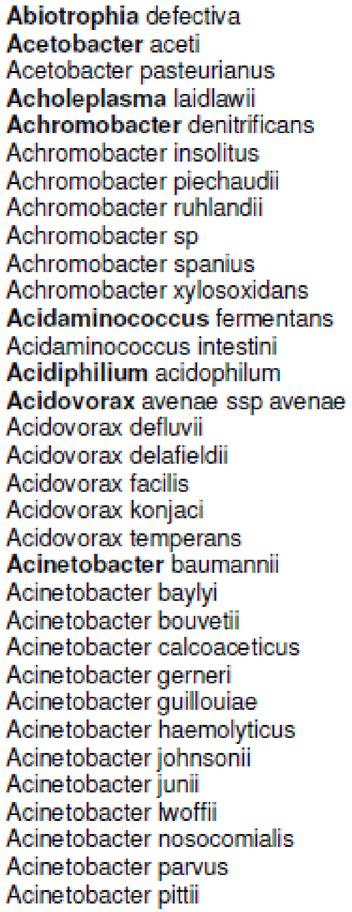
● MSPs from Taxonomy Trees

Taxonomy
Bruker Taxonomy
Projects

3/ Select the parameters (processing and identification methods set by Bruker)

Biotyper workflow

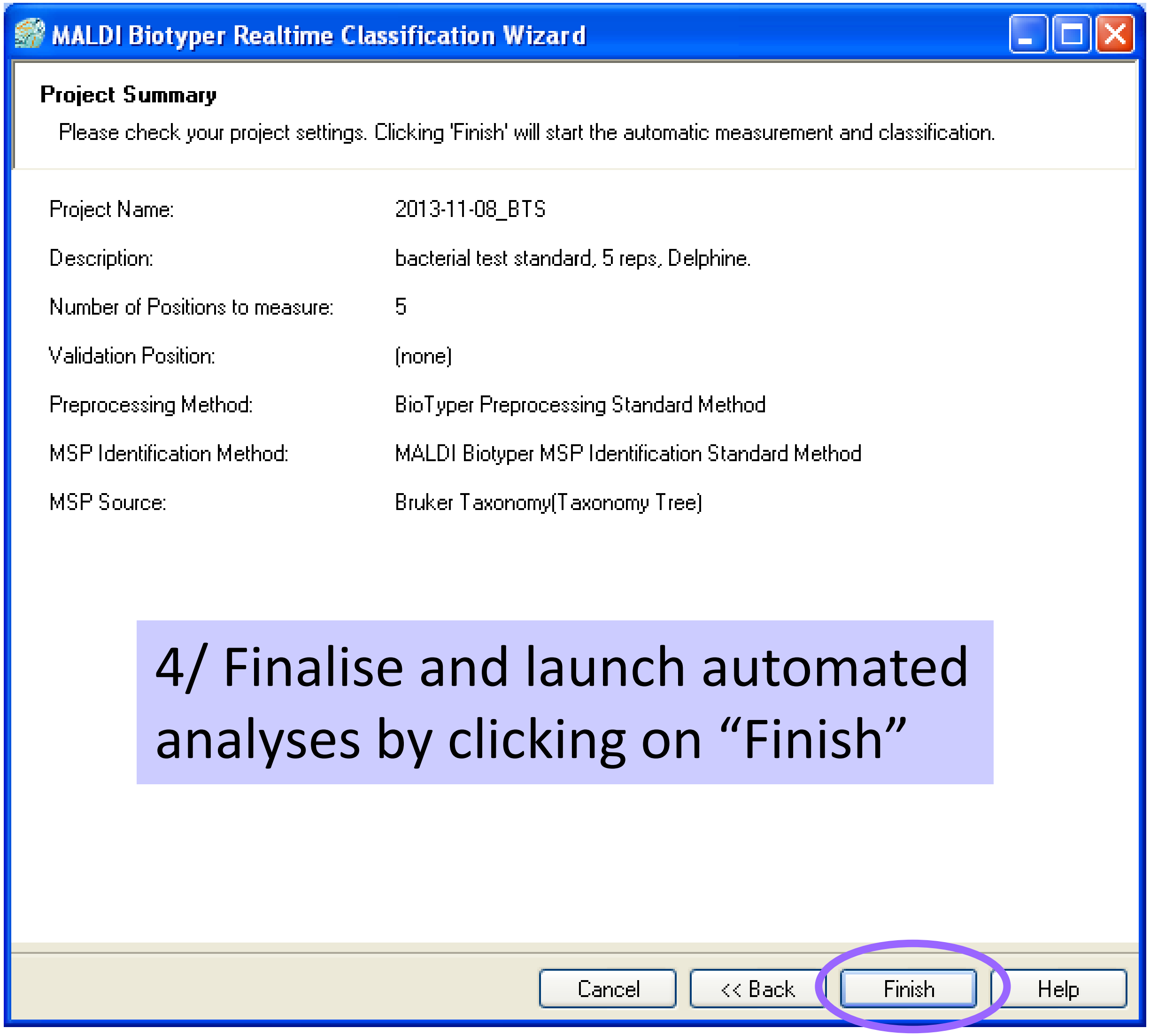
DB 4613: complete genus/species list



Abiotrophia defectiva
Acetobacter aceti
Acetobacter pasteurianus
Acholeplasma laidlawii
Achromobacter denitrificans
Achromobacter insolitus
Achromobacter piechaudii
Achromobacter ruhlandii
Achromobacter sp
Achromobacter spanius
Achromobacter xylosoxidans
Acidaminococcus fermentans
Acidaminococcus intestini
Acidiphilium acidophilum
Acidovorax avenae ssp avenae
Acidovorax defluvii
Acidovorax delafieldii
Acidovorax facilis
Acidovorax konjaci
Acidovorax temperans
Acinetobacter baumannii
Acinetobacter baylyi
Acinetobacter bouvetii
Acinetobacter calcoaceticus
Acinetobacter gerneri
Acinetobacter guillouiae
Acinetobacter haemolyticus
Acinetobacter johnsonii
Acinetobacter junii
Acinetobacter lwoffii
Acinetobacter nosocomialis
Acinetobacter parvus
Acinetobacter pittii

Brucker Taxonomy database contains 4613 species of bacteria

Biotyper workflow



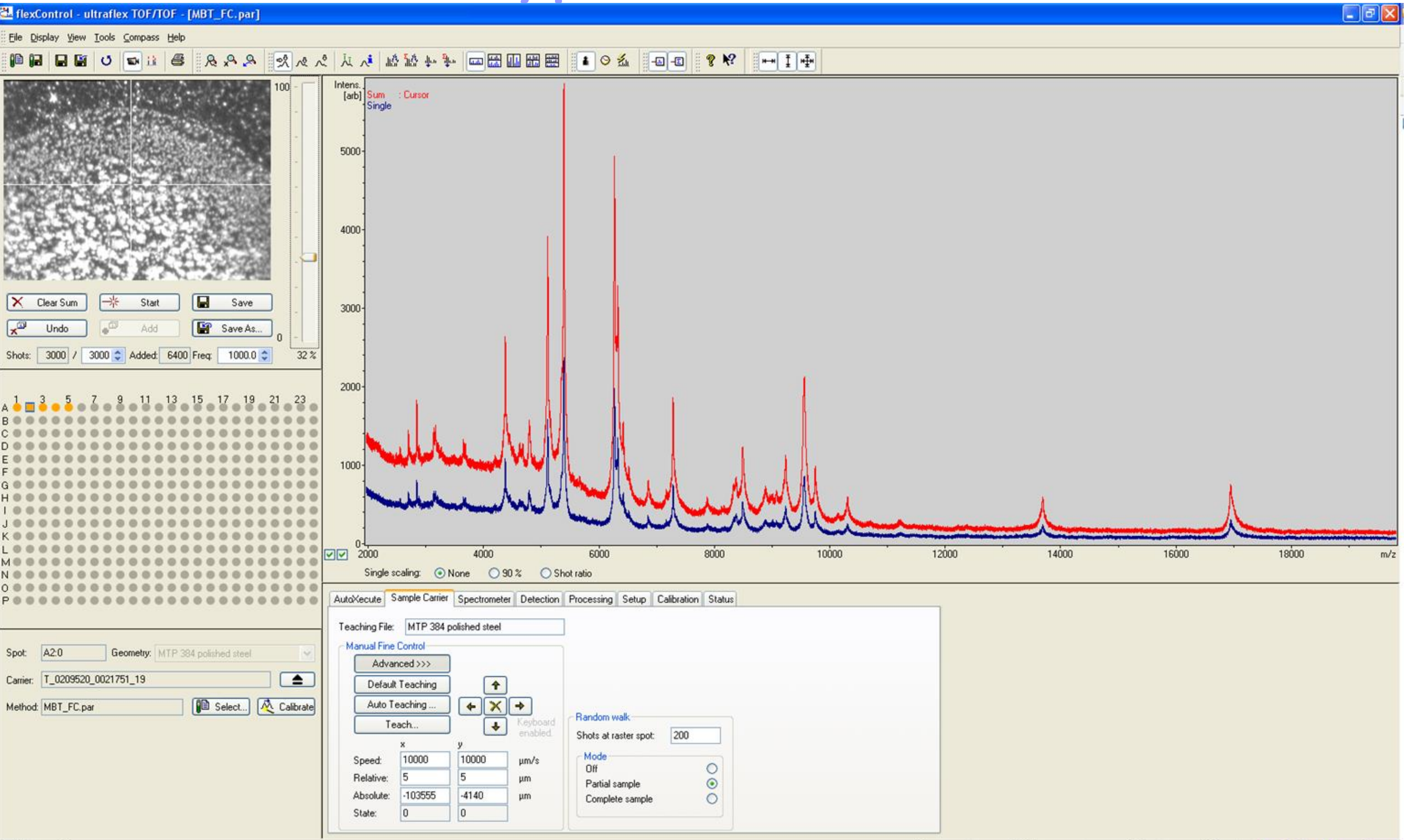
Project Summary

Please check your project settings. Clicking 'Finish' will start the automatic measurement and classification.

Project Name:	2013-11-08_BTS
Description:	bacterial test standard, 5 reps, Delphine.
Number of Positions to measure:	5
Validation Position:	(none)
Preprocessing Method:	BioTyper Preprocessing Standard Method
MSP Identification Method:	MALDI Biotyper MSP Identification Standard Method
MSP Source:	Bruker Taxonomy(Taxonomy Tree)

4/ Finalise and launch automated analyses by clicking on “Finish”

Biotyper workflow



flexControl - ultraflex TOF/TOF - [MBT_FC.par]

Shots: 3000 / 3000 Added: 6400 Freq: 1000.0

Single scaling: ● None ○ 90 % ○ Shot ratio

Spot: A2:0 Geometry: MTP 384 polished steel

Carrier: T_0209520_0021751_19

Method: MBT_FC.par

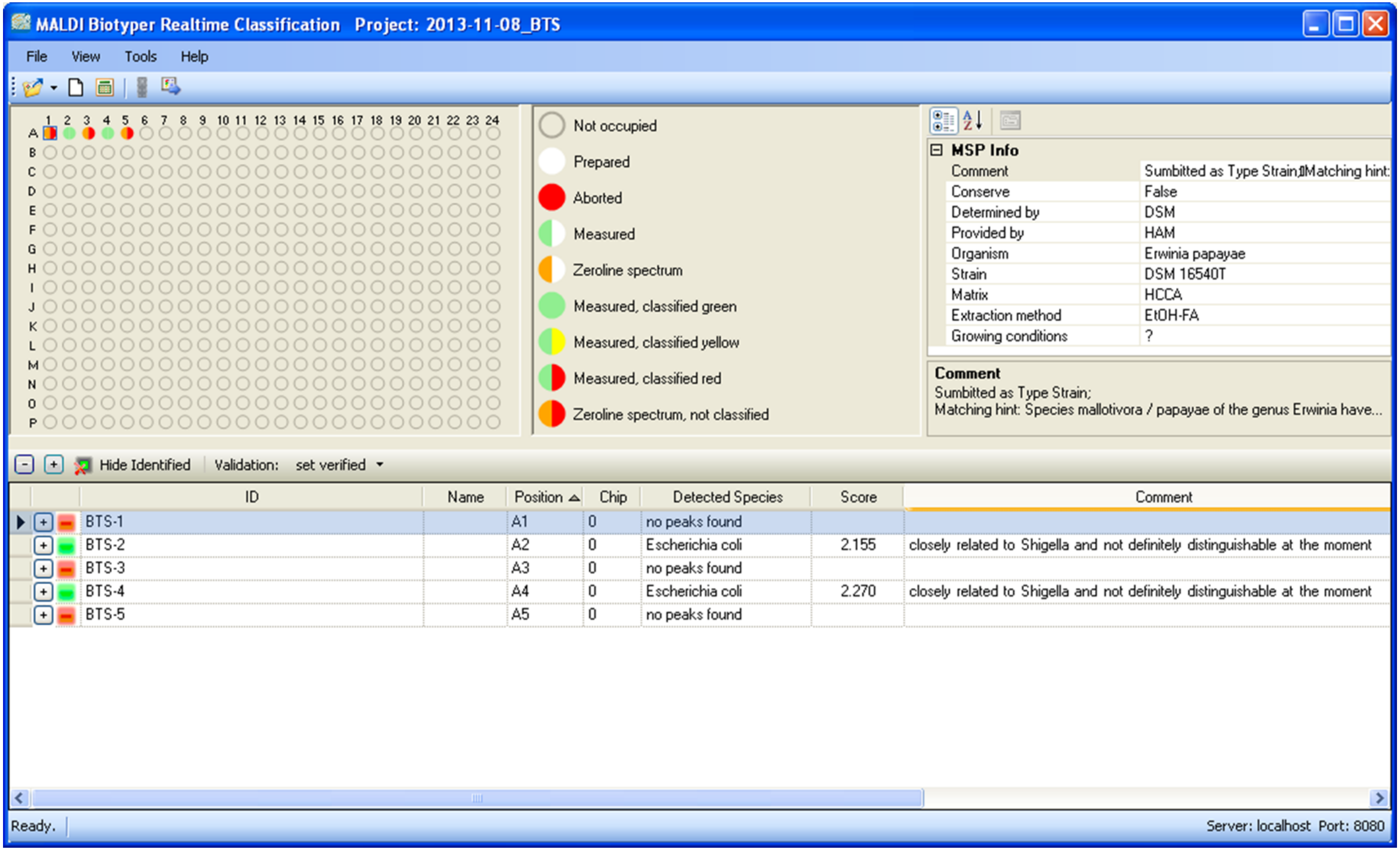
Teaching File: MTP 384 polished steel

	x	y	
Speed:	10000	10000	µm/s
Relative:	5	5	µm
Absolute:	-103555	-4140	µm
State:	0	0	

Shots at raster spot: 200

Mode
- Off ○
- Partial sample ●
- Complete sample ○

Biotyper workflow



- ○ Not occupied
- ○ Prepared
- 🔴 Aborted
- ◐ Measured
- ◐ Zeroline spectrum
- 🟢 Measured, classified green
- ◐ Measured, classified yellow
- ◐ Measured, classified red
- ◐ Zeroline spectrum, not classified

MSP Info

Comment	Sumbitted as Type Strain;Matching hint:
Conserve	False
Determined by	DSM
Provided by	HAM
Organism	Erwinia papayae
Strain	DSM 16540T
Matrix	HCCA
Extraction method	EtOH-FA
Growing conditions	?

Comment

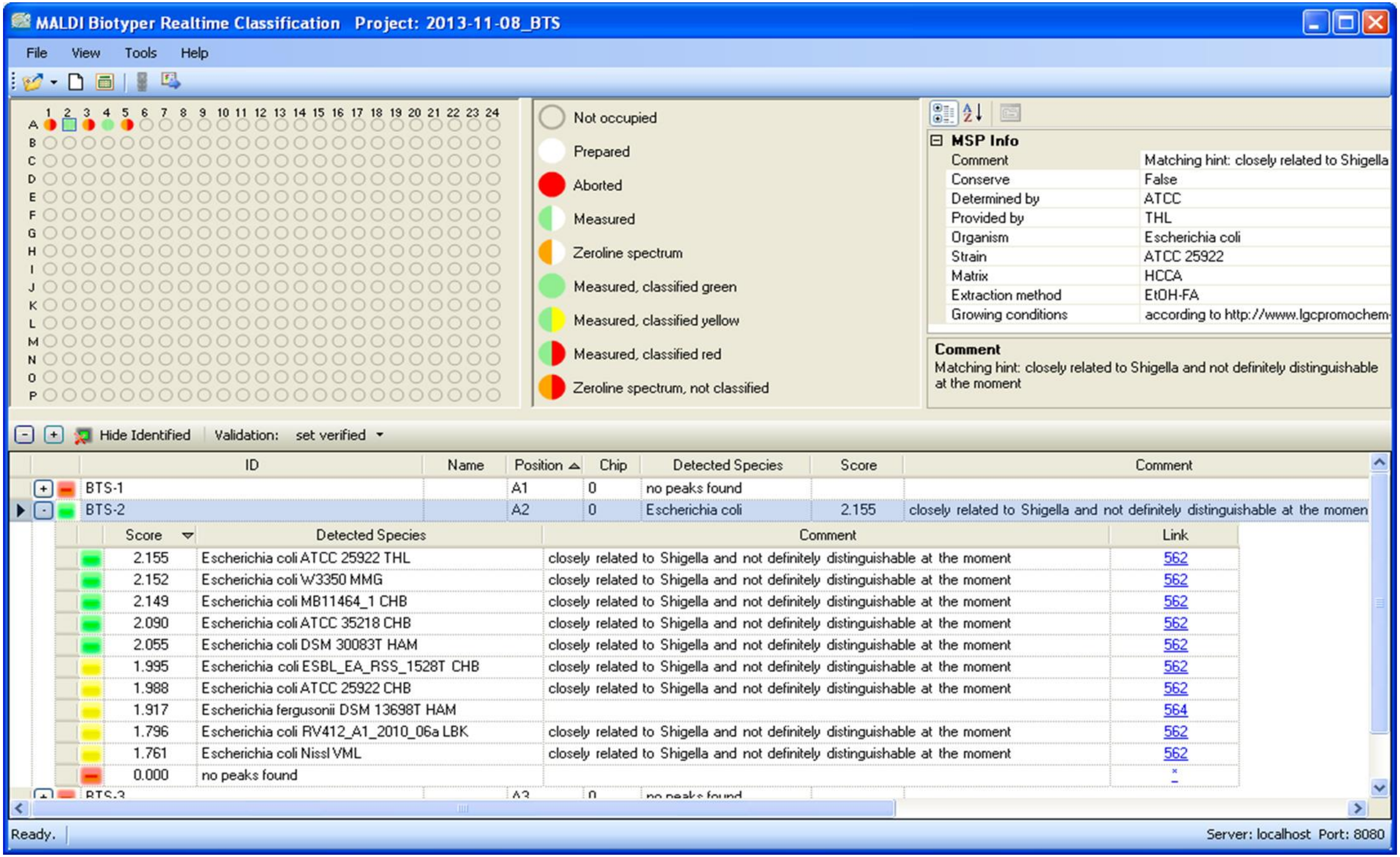
Sumbitted as Type Strain;
Matching hint: Species mallotivora / papayae of the genus Erwinia have...

	ID	Name	Position	Chip	Detected Species	Score	Comment
🟥	BTS-1		A1	0	no peaks found		
🟩	BTS-2		A2	0	Escherichia coli	2.155	closely related to Shigella and not definitely distinguishable at the moment
🟥	BTS-3		A3	0	no peaks found		
🟩	BTS-4		A4	0	Escherichia coli	2.270	closely related to Shigella and not definitely distinguishable at the moment
🟥	BTS-5		A5	0	no peaks found		

Ready.

Server: localhost Port: 8080

Biotyper workflow



- ○ Not occupied
- ○ Prepared
- 🔴 Aborted
- ◐ Measured
- ◐ Zeroline spectrum
- 🟢 Measured, classified green
- ◐ Measured, classified yellow
- ◐ Measured, classified red
- ◐ Zeroline spectrum, not classified

MSP Info

Comment	Matching hint: closely related to Shigella
Conserve	False
Determined by	ATCC
Provided by	THL
Organism	Escherichia coli
Strain	ATCC 25922
Matrix	HCCA
Extraction method	EtOH-FA
Growing conditions	according to http://www.lgcpromochem

Comment
Matching hint: closely related to Shigella and not definitely distinguishable at the moment

	ID	Name	Position	Chip	Detected Species	Score	Comment
+ 🟥	BTS-1		A1	0	no peaks found		
- 🟩	BTS-2		A2	0	Escherichia coli	2.155	closely related to Shigella and not definitely distinguishable at the momen

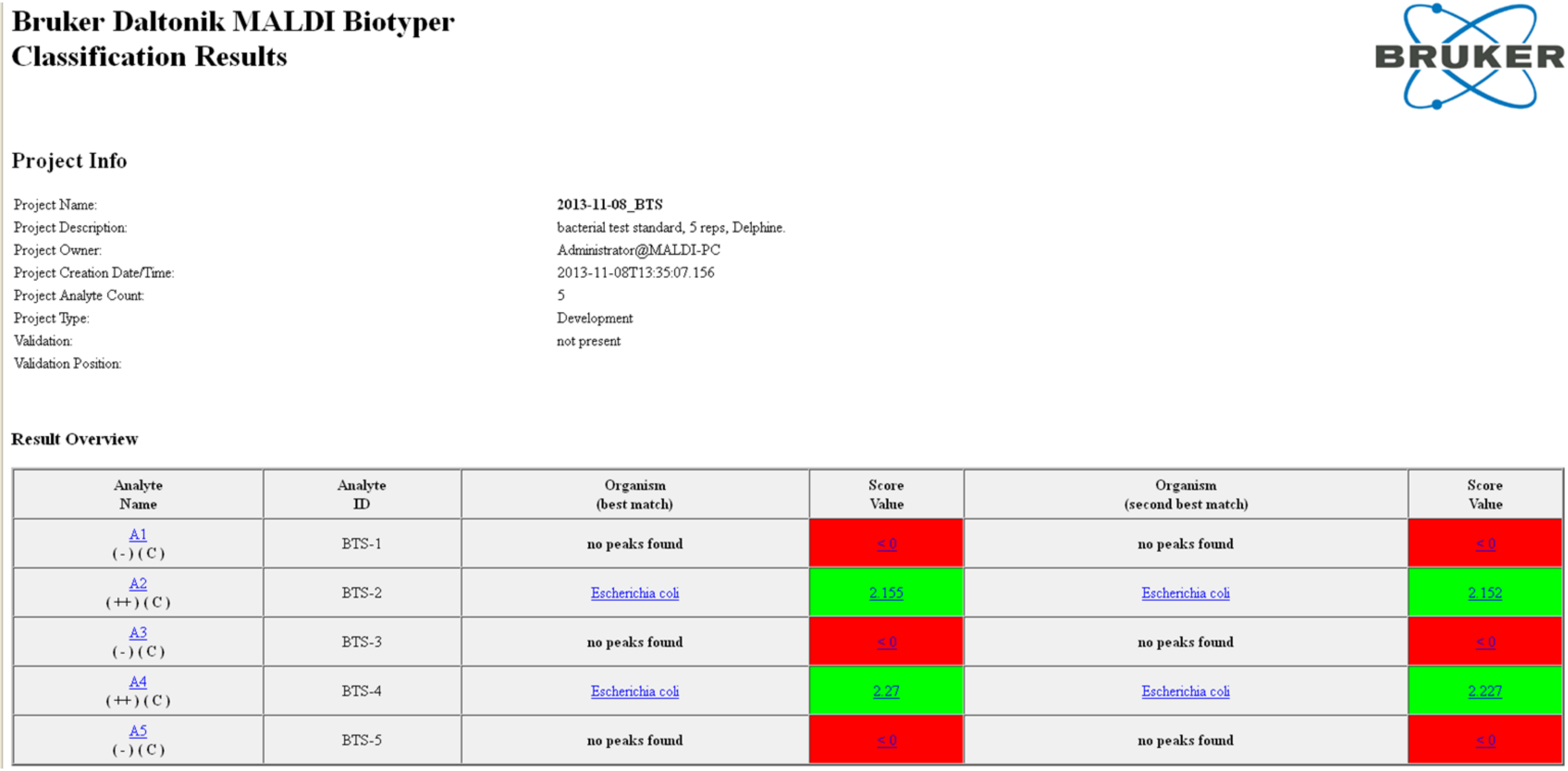
	Score	Detected Species	Comment	Link
🟩	2.155	Escherichia coli ATCC 25922 THL	closely related to Shigella and not definitely distinguishable at the moment	562
🟩	2.152	Escherichia coli W3350 MMG	closely related to Shigella and not definitely distinguishable at the moment	562
🟩	2.149	Escherichia coli MB11464_1 CHB	closely related to Shigella and not definitely distinguishable at the moment	562
🟩	2.090	Escherichia coli ATCC 35218 CHB	closely related to Shigella and not definitely distinguishable at the moment	562
🟩	2.055	Escherichia coli DSM 30083T HAM	closely related to Shigella and not definitely distinguishable at the moment	562
🟨	1.995	Escherichia coli ESBL_EA_RSS_1528T CHB	closely related to Shigella and not definitely distinguishable at the moment	562
🟨	1.988	Escherichia coli ATCC 25922 CHB	closely related to Shigella and not definitely distinguishable at the moment	562
🟨	1.917	Escherichia fergusonii DSM 13698T HAM		564
🟨	1.796	Escherichia coli RV412_A1_2010_06a LBK	closely related to Shigella and not definitely distinguishable at the moment	562
🟨	1.761	Escherichia coli Nissl VML	closely related to Shigella and not definitely distinguishable at the moment	562
🟥	0.000	no peaks found		×

	ID	Name	Position	Chip	Detected Species	Score	Comment
+ 🟥	BTS-3		A3	0	no peaks found		

Ready.

Server: localhost Port: 8080

Biotyper workflow



Bruker Daltonik MALDI Biotyper Classification Results

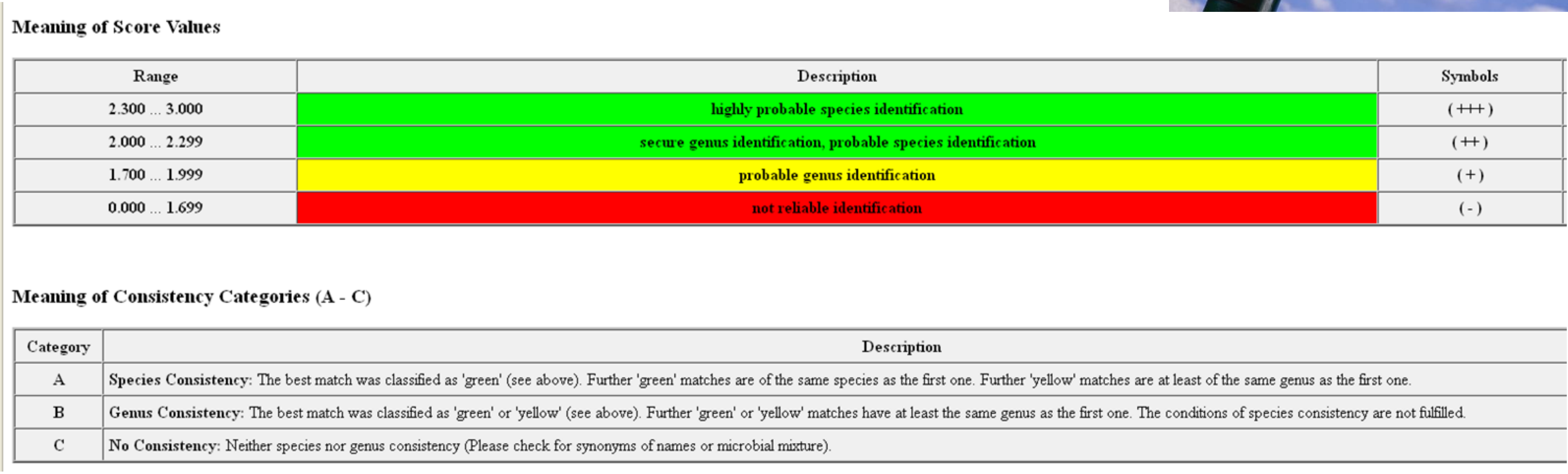
Project Info

Project Name:	**2013-11-08_BTS**
Project Description:	bacterial test standard, 5 reps, Delphine.
Project Owner:	Administrator@MALDI-PC
Project Creation Date/Time:	2013-11-08T13:35:07.156
Project Analyte Count:	5
Project Type:	Development
Validation:	not present
Validation Position:	

Result Overview

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
A1 (-) (C)	BTS-1	**no peaks found**	< 0	**no peaks found**	< 0
A2 (++) (C)	BTS-2	Escherichia coli	2.155	Escherichia coli	2.152
A3 (-) (C)	BTS-3	**no peaks found**	< 0	**no peaks found**	< 0
A4 (++) (C)	BTS-4	Escherichia coli	2.27	Escherichia coli	2.227
A5 (-) (C)	BTS-5	**no peaks found**	< 0	**no peaks found**	< 0

Biotyper workflow



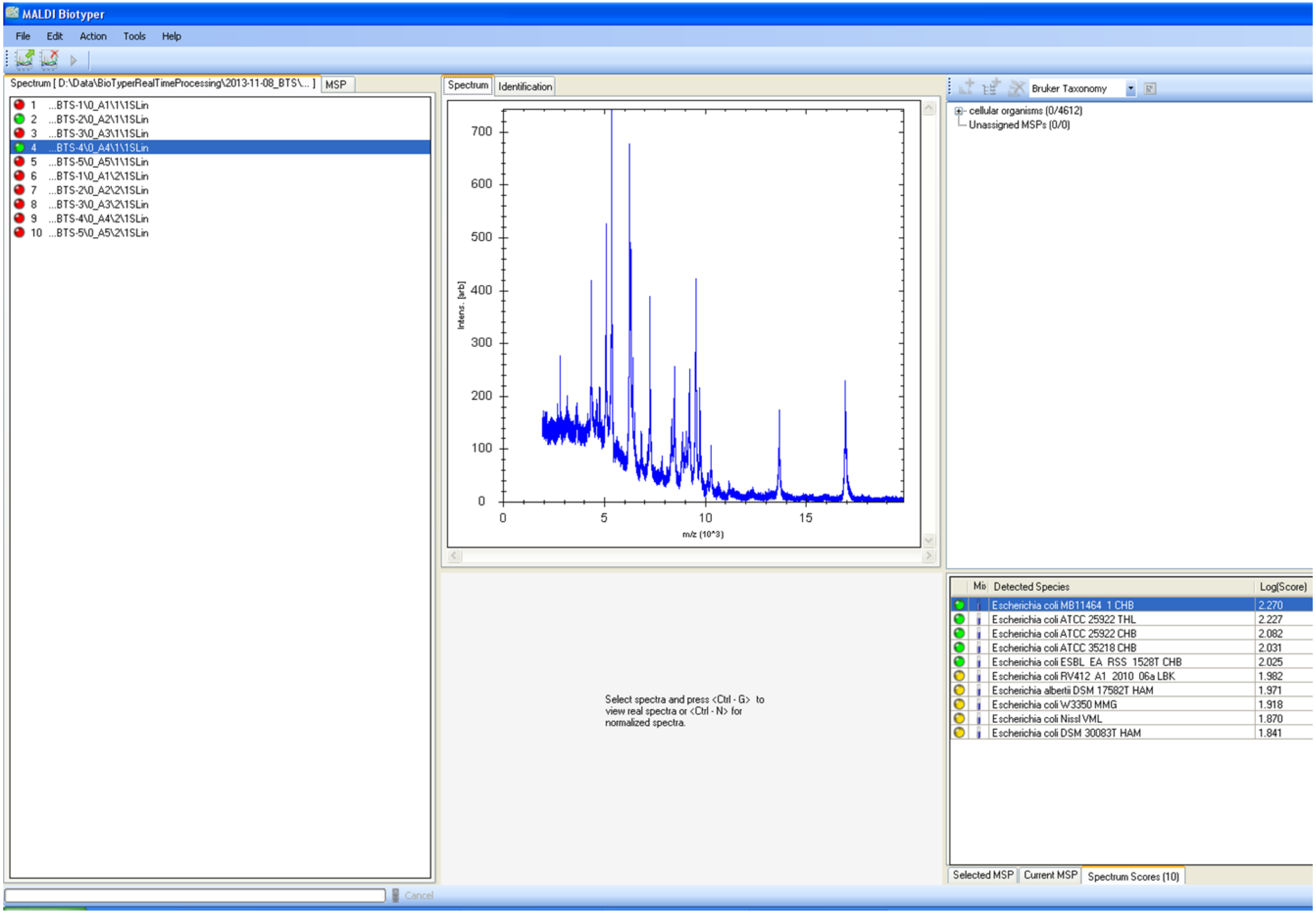
Meaning of Score Values

Range	Description	Symbols
2.300 ... 3.000	🟩 highly probable species identification	(+++)
2.000 ... 2.299	🟩 secure genus identification, probable species identification	(++)
1.700 ... 1.999	🟨 probable genus identification	(+)
0.000 ... 1.699	🟥 not reliable identification	(-)

Meaning of Consistency Categories (A - C)

Category	Description
A	**Species Consistency:** The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.
B	**Genus Consistency:** The best match was classified as 'green' or 'yellow' (see above). Further 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
C	**No Consistency:** Neither species nor genus consistency (Please check for synonyms of names or microbial mixture).

Biotyper workflow

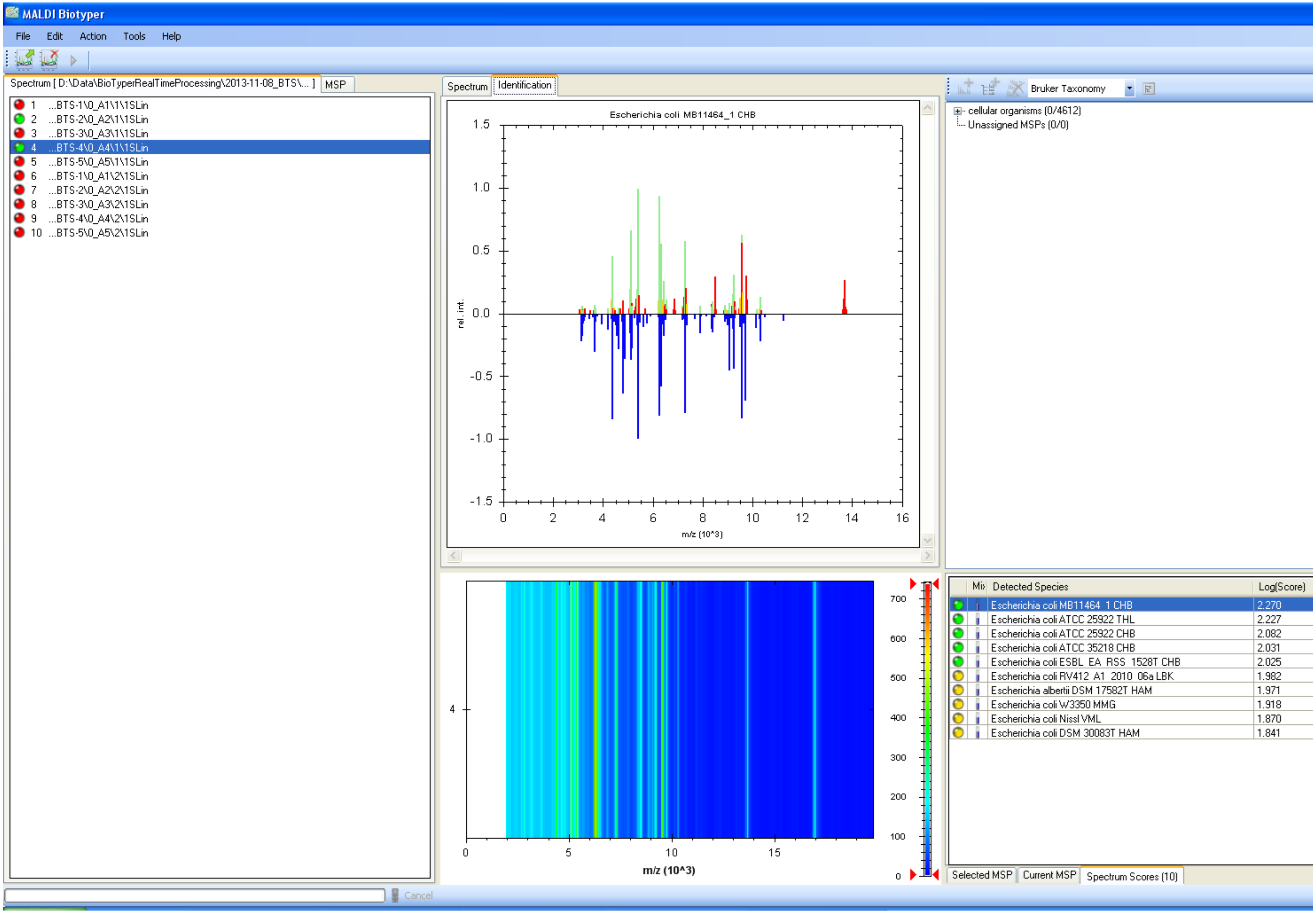


🔴	1	...BTS-1\0_A1\1\1SLin
🟢	2	...BTS-2\0_A2\1\1SLin
🔴	3	...BTS-3\0_A3\1\1SLin
🟢	4	...BTS-4\0_A4\1\1SLin
🔴	5	...BTS-5\0_A5\1\1SLin
🔴	6	...BTS-1\0_A1\2\1SLin
🔴	7	...BTS-2\0_A2\2\1SLin
🔴	8	...BTS-3\0_A3\2\1SLin
🔴	9	...BTS-4\0_A4\2\1SLin
🔴	10	...BTS-5\0_A5\2\1SLin

Select spectra and press <Ctrl - G> to view real spectra or <Ctrl - N> for normalized spectra.

	Mi:	Detected Species	Log(Score)
🟢		Escherichia coli MB11464 1 CHB	2.270
🟢		Escherichia coli ATCC 25922 THL	2.227
🟢		Escherichia coli ATCC 25922 CHB	2.082
🟢		Escherichia coli ATCC 35218 CHB	2.031
🟢		Escherichia coli ESBL EA RSS 1528T CHB	2.025
🟡		Escherichia coli RV412 A1 2010 06a LBK	1.982
🟡		Escherichia albertii DSM 17582T HAM	1.971
🟡		Escherichia coli W3350 MMG	1.918
🟡		Escherichia coli Nissl VML	1.870
🟡		Escherichia coli DSM 30083T HAM	1.841

Biotyper workflow



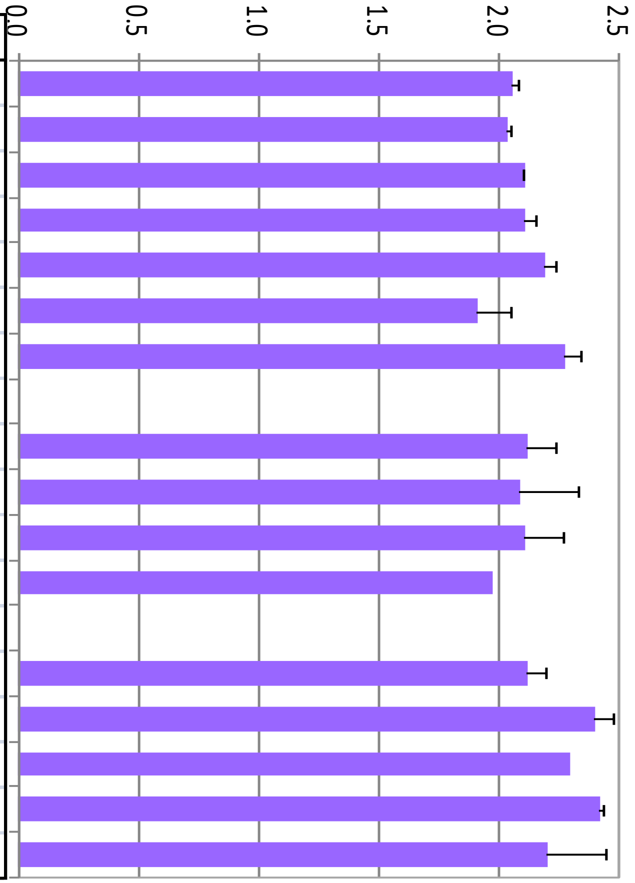
Spectrum [D:\Data\BioTyperRealTimeProcessing\2013-11-08_BTS\...]

- 🔴 1 ...BTS-1\0_A1\1\1SLin
- 🟢 2 ...BTS-2\0_A2\1\1SLin
- 🔴 3 ...BTS-3\0_A3\1\1SLin
- 🟢 4 ...BTS-4\0_A4\1\1SLin
- 🔴 5 ...BTS-5\0_A5\1\1SLin
- 🔴 6 ...BTS-1\0_A1\2\1SLin
- 🔴 7 ...BTS-2\0_A2\2\1SLin
- 🔴 8 ...BTS-3\0_A3\2\1SLin
- 🔴 9 ...BTS-4\0_A4\2\1SLin
- 🔴 10 ...BTS-5\0_A5\2\1SLin

	Mi:	Detected Species	Log(Score)
🟢		Escherichia coli MB11464_1 CHB	2.270
🟢		Escherichia coli ATCC 25922 THL	2.227
🟢		Escherichia coli ATCC 25922 CHB	2.082
🟢		Escherichia coli ATCC 35218 CHB	2.031
🟢		Escherichia coli ESBL_EA_RSS_1528T CHB	2.025
🟡		Escherichia coli RV412_A1_2010_06a LBK	1.982
🟡		Escherichia albertii DSM 17582T HAM	1.971
🟡		Escherichia coli W3350 MMG	1.918
🟡		Escherichia coli Nissl VML	1.870
🟡		Escherichia coli DSM 30083T HAM	1.841

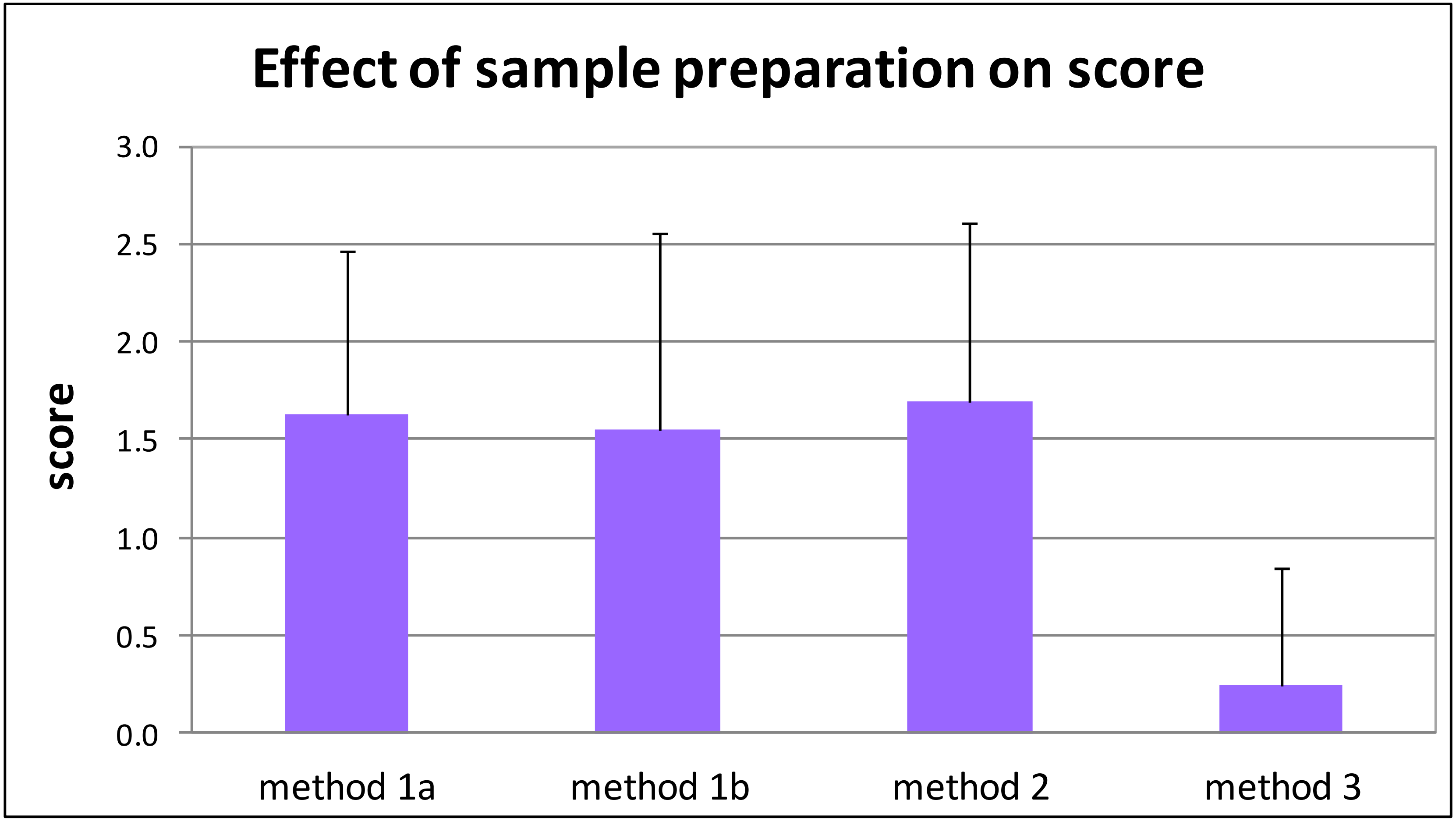
Results

no.	reference species	correct ID	mean	SD
1	Acinetobacter johnsonii	YES	2.061	0.034
2	Bacillus cereus	YES	2.041	0.023
3	Bacillus thuringiensis	YES	2.116	0.000
4	Bacteroides vulgatus	YES	2.107	0.061
5	Campylobacter fetus	YES	2.194	0.053
6	Citrobacter freundii	YES	1.919	0.140
7	Clostridium septicum	YES	2.277	0.079
8	Clostridium tertium	no ID	0.000	
9	Enterobacter asburiae	YES	2.118	0.130
10	Erysipelothrix rhusiopathiae	YES	2.094	0.251
11	Escherichia coli	YES	2.114	0.167
12	Mannheimia haemolytica	YES	1.976	
13	Mycoplasma	no ID	0.000	
14	Pasteurella multocida	YES	2.128	0.081
15	Staphylococcus aureus	YES	2.399	0.084
16	Staphylococcus chromogenes	YES	2.298	
17	Streptococcus dysgalactiae	YES	2.422	0.023
18	Yersinia pseudotuberculosis	YES	2.202	0.259



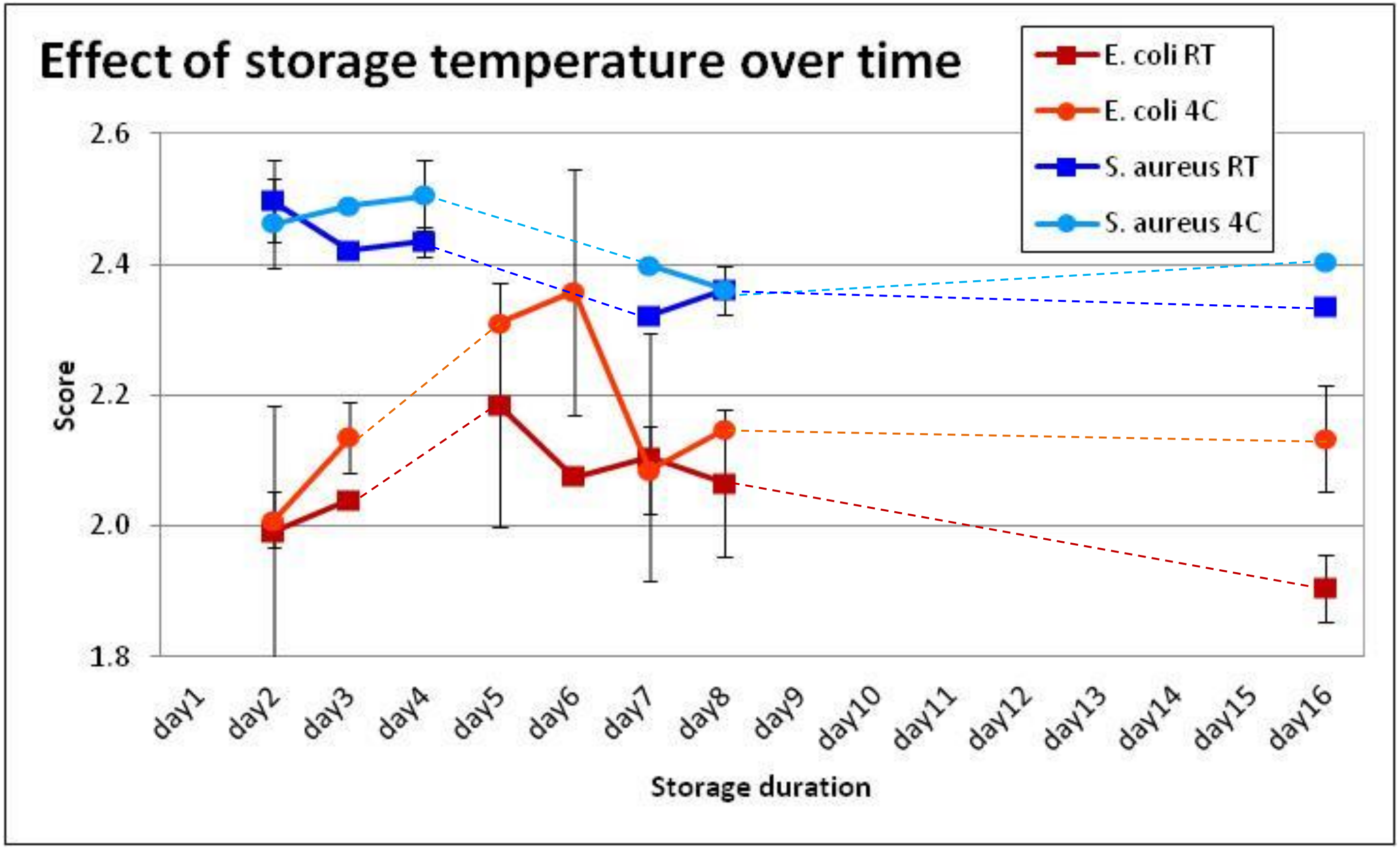
16/18 species from the reference set were successfully identified, thus confirming the biochemical assay results.

Results



Methods 1a (direct transfer), 1b (DT + FA) and 2 (EtOH/FA) are comparable. Method 3 (TFA) gave no identification results.

Results



Scores are higher for S. aureus than for E. coli.
Scores are slightly better when plate are stored at 4C.
Scores seem to decrease as culture age.

Conclusions

- Could Biotyper become a routine technique to identify bacteria from animal tissues?
  - MALDI Biotyper reliably identifies bacteria at the species level (16/18 species from the reference set).
  - Method 1 and 2 gives best scores. Method 1 is much simpler and time/cost-efficient.
  - Storage conditions (4°C or RT) and age of culture plate have a negligible effect on results.
  - It's much quicker, easier, cheaper than the traditional methods

YES, it could!

Future directions

- What’s next?
  - Complete Biotyper assessment using the rest of the reference set.
  - Futher validate the strategy on “real” samples (from animal –Simone Warner-, or plant –Brendan Rodoni- tissues).
  - Beside bacteria, attempt identification of filamentous fungi and mycobacteria (need to purchase the database).
  - An honour’s student might be hired for this.