RESEARCH ARTICLE

Evaluation of repetitive-PCR and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for rapid strain typing of *Bacillus coagulans*

Jun Sato1,2*, Motokazu Nakayama3, Ayumi Tomita1, Takumi Sonoda1, Motomitsu Hasumi1, Takahisa Miyamoto4

1 Safety Science Research, R&D, Kao Corporation, Ichikai, Tochigi, Japan, 2 Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Hakozaki, Higashi-ku, Fukuoka, Japan, 3 Health Care Food Research, R&D, Kao Corporation, Sumida, Tokyo, Japan, 4 Division of Food Science & Biotechnology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka, Japan

* satou.jun5@kao.co.jp

Abstract

In order to establish rapid and accurate typing method for *Bacillus coagulans* strains which is important for controlling in some canned foods and tea-based beverages manufacturing because of the high-heat resistance of the spores and high tolerance of the vegetative cells to catechins and chemicals, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and repetitive-PCR (rep-PCR) were evaluated. For this purpose, 28 strains of *B. coagulans* obtained from various culture collections were tested. DNA sequence analyses of the genes encoding 16S rRNA and DNA gyrase classified the test strains into two and three groups, respectively, regardless of their phenotypes. Both MALDI-TOF MS and rep-PCR methods classified the test strains in great detail. Strains classified in each group showed similar phenotypes, such as carbohydrate utilization determined using API 50CH. In particular, the respective two pairs of strains which showed the same metabolic characteristic were classified into the same group by both MALDI-TOF MS and rep-PCR methods separating from the other strains. On the other hand, the other strains which have the different profiles of carbohydrate utilization were separated into different groups by these methods. These results suggested that the combination of MALDI-TOF MS and rep-PCR analyses was advantageous for the rapid and detailed typing of bacterial strains in respect to both phenotype and genotype.

Introduction

Rapid identification of bacterial species and typing of strains are important for the assessment of clonal relationships among environmental isolates in food and beverage processing industries, especially when bacterial spoilage occurred on their products. The conventional methods...
of bacterial analysis, however, require high expertise, great experience, and long time for precise identification and typing. As a result of the development of the polymerase chain reaction (PCR) method in the 1990’s, molecular biological methods for identification of prokaryotes were established on the basis of the nucleotide sequences of 16S ribosomal RNA gene [1]. This bacterial identification technique spread widely in order to improve the bacterial control in food and beverage manufacturing. Evaluation of bacterial species could now be achieved within a day using this technique. Rapid typing methods of bacterial strains are needed to identify the origin of the spoilage bacteria as well as the identification of bacterial species. As nucleic acid-based typing methods, multilocus sequence typing (MLST) [2], pulse-field gel electrophoresis (PFGE) [3], random amplified polymorphic DNA (RAPD) [4], and Ribotyping [5, 6], are well known. Although MLST and PFGE have sufficient discrimination, these methods are time-consuming and labor intensive, requiring 2 working days for sample preparation and analysis. The RAPD method is capable of obtaining the results of strain typing within half a day. However, the reproducibility of band patterns obtained by amplification by arbitrarily primed PCR using short-length primers needs to be confirmed [7, 8]. In recent years, an automated microbial typing system with repetitive sequence-based PCR (rep-PCR) [9–11] and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) [12, 13] have been used for identification of bacterial species and may also provide potential alternative methods for strain typing. The genus Bacillus is also important target of strain typing for both clinical and industrial fields because of including pathogenic or food spoilage bacteria species such as Bacillus anthrasis, B. cereus, B. licheniformis, and B. subtilis [14, 15]. MALDI-TOF fingerprint and rep-PCR had potential to be obtained valuable information at both intra- and interspecies levels in the Bacillus species in previous study [14, 16]. Especially we focused on Bacillus coagulans which is well-known as a causative agent of flat-sour spoilage in some canned foods and tea-based beverages manufacturing [17, 18] as well as a representative bacterium for probiotics by lactic acid production [19]. In this study, we evaluated the reliability of rep-PCR and MALDI-TOF MS as strain typing of Bacillus coagulans by the comparison of ribotyping and phenotype of carbohydrate utilization.

Materials and methods

Bacterial strains and culture

Twenty-eight strains of B. coagulans were obtained from the National Institute of Technology and Evaluation (NITE), Japan Canner Association (JCA), and Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) (Table 1). The type strain of B. coagulans NBRC12583T was purchased from the NITE Biological Resource Center. Eighteen JCA strains were the isolates from various spoiled food products.

B. coagulans strains were cultured on soybean-casein digested agar (Wako, Osaka, Japan) at 45˚C for 2 days, and the absence of spores was confirmed by phase contrast microscopy.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Single colony was picked up from an agar plate and suspended in 300 μL distilled water. The cell suspension was mixed with 900 μL ethanol (Wako Pure Chemicals Inc., Osaka, Japan), and centrifuged at 13,000 rpm for 2 min at room temperature. The supernatant was discarded and residual ethanol was allowed to evaporate completely. Pellets were resuspended in 30 μL of 70% formic acid (Wako, Osaka, Japan), mixed with 30 μL acetonitrile (Wako Pure Chemicals Inc., Osaka, Japan) and centrifuged at 13,000 rpm for 2 min at room temperature. After a
centrifugation, 1 μL of the supernatant was spotted onto a disposable steel plate (SYSMEX bioMérieux, Tokyo, Japan) and air dried at room temperature. Each sample was spotted three replicates on the plates. Sample spots were overlaid with 1 μL of matrix solution (α-cyano-4-hydroxy cinnamic acid; SYSMEX bioMérieux, Tokyo, Japan) and air dried at room temperature. The mass spectra were obtained using an AXIMA performance MALDI-TOF MS (Shimadzu Corporation, Kyoto, Japan) with a nitrogen laser (λ = 337.1 nm) and a mass range m/z from 3000 to 20,000. Shimadzu Biotech Launchpad software (Shimadzu Corporation, Kyoto, Japan) was used for mass spectra acquisition and peak detection. The calibration of mass spectra was performed using *Escherichia coli* every analysis. Cluster analysis of mass spectra was performed with SARAMIS software (database V4.10, Vitek MS plus; SYSMEX bioMérieux, Tokyo, Japan) to discriminate *B. coagulans* strains. A dendrogram was made with single link agglomerative clustering algorithm based on mass spectra. The strains having the mass spectra with more than 80% in similarity were defined as the same strain.

Table 1. *Bacillus coagulans* strains used in this study.

| No. | Strain No. | Source |
|-----|------------|--------|
| 1   | *NBRC12583*<sup>T</sup> | Evaporated milk |
| 2   | *JCA1108* | Spoiled canned mushrooms in spring water |
| 3   | JCA1109 |  |
| 4   | JCA1111 | Canned curry |
| 5   | JCA1115 | Canned crab meat |
| 6   | JCA1116 | Canned squid |
| 7   | JCA1117 |  |
| 8   | JCA1118 | Curry before canned |
| 9   | JCA1120 | Canned sausage |
| 10  | JCA1121 |  |
| 11  | JCA1122 | Canned flavored squid |
| 12  | JCA1131 | Canned tuna |
| 13  | JCA1158 | Canned mackerel in oil |
| 14  | JCA1174 | Canned green peas in spring water |
| 15  | JCA1175 |  |
| 16  | JCA1177 | Canned flavored arch clams |
| 17  | JCA1178 |  |
| 18  | JCA1180 | Spoiled retort pouch food |
| 19  | JCA1182 | Spoiled retort pouch sauce |
| 20  | *DSM2308* |  |
| 21  | DSM2311 |  |
| 22  | DSM2312 |  |
| 23  | DSM2314 | Rhizosphere |
| 24  | DSM2350 |  |
| 25  | DSM2356 |  |
| 26  | DSM2383 |  |
| 27  | DSM2384 |  |
| 28  | DSM2385 |  |

*: Unknown

<sup>1</sup> NITE Biological Resource Centre

<sup>2</sup> Japan Canner Association

<sup>3</sup> Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

https://doi.org/10.1371/journal.pone.0186327.t001
Strain typing using the repetitive sequence-based PCR method

Genomic DNA was extracted and purified using an UltraClean Microbial DNA Isolation kit (MO BIO, CA, USA) from the vegetative cells after the cultivation. DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher, MA, USA). Purified DNA was diluted to 25–50 ng/μL with ultrapure water. Rep-PCR was performed using the DiversiLab Bacillus kit (SYSMEX bioMérieux, Tokyo, Japan) according to the manufacturer’s protocol. The PCR products were analyzed using DiversiLab using microfluidics chip technology with an Agilent 2100 Bioanalyzer (Agilent Technologies, Tokyo, Japan). The data from DiversiLab for each strain consisted of a visualized fluorescence intensity corresponding to a band pattern, a dendrogram from an unweighted pair group method with arithmetic mean (UPGMA), and a similarity matrix between each strain. The strains having more than 95% in similarity on the basis of the data from DiversiLab were grouped into the same group. The strains having similarity more than 99% were regarded as an identical strain. Independent experiment was repeated three times.

Ribotyping

The ribotypes of the strains were determined by using a RiboPrinter™ Microbial Characterization System (DuPont, DE, USA) with the restriction enzyme, EcoRI. This automated ribotyping system includes each step, such as bacterial cell lysis, restriction enzyme cleavage, electrophoresis, blotting, and detection [20]. The strains having similarity more than 0.9 were regarded as a same ribogroup.

Comparison of physiological properties using API 50 CH

The carbohydrate utilization of the bacterial strains was determined by using an API 50CH system (SYSMEX bioMérieux, Tokyo, Japan) according to the manufacturer’s instructions. Color changes were confirmed after incubation for 24 and 48 h at 45°C.

DNA sequencing of 16S rRNA and gyrB

Parts of the 16S rDNA and gyrB genes were amplified from the genomic DNAs prepared from each of the bacterial strains by PCR using the primers described previously [21]. Multiple sequence alignment of 16S rRNA and gyrB was performed using open source software ClustalX2 V2.1 (http://www.clustal.org/clustal2/), after adjusting the length of nucleotide sequence of each strain with Seaview (http://doua.prabi.fr/software/seaview). Bootstrap values were calculated by the neighbor-joining method [22] using ClustalX2. The phylogenetic tree based on the ClustalX2 analysis was displayed using open-source NJplot software (http://doua.prabi.fr/software/njplot).

Ribosomal internal transcribed spacer region analysis

Polymorphism analysis of the internal transcribed spacer (ITS) region of the 16S-23S ribosomal DNA sequence was performed according to the method of Daffonchio et al. [23]. The template DNA was prepared from each of the strains using Prepman Ultra (Applied Biosystems, CA, USA). PCR was done with TaKaRa ExTaq Hot Start Version (Takara Bio, Shiga, Japan), template DNA, and the primers in a reaction volume of 50 μL. The PCR conditions were initial denaturation at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and final extension at 72°C for 2 min.
Results
Phylogenetic analysis of *B. coagulans* strains based on DNA sequence of 16S rRNA and *gyrB*

Phylogenetic trees derived from the 16S rRNA gene (1458 bp) and *gyrB* (1179 bp) sequences separated into two and three clusters, respectively (Fig 1). Six strains, DSM2311, DSM2312, DSM2314, JCA1120, JCA1121, and JCA1158, were clearly separated from the major cluster for both genes. Other strains were included in major cluster I of both genes. Although *gyrB* gene sequencing has been used for phylogenetic study of genus *Bacillus* [21] because its evolutionary speed is faster than 16S rRNA gene, it was difficult to distinguish *B. coagulans* strains using these housekeeping gene sequence analysis.

![Phylogenetic trees of *Bacillus coagulans* strains based on nucleotide sequences of (A) 16S rRNA and (B) *gyrB*. The trees were constructed using the neighbor-joining method, and genetic distances were computed using Kimura’s model. Numbers at the nodes indicate percentages of 1000 bootstrapped trees. The bars indicate genetic distances. *Bacillus subtilis* NBRC13419T and 168 strains were used as the outgroups for phylogenetic analyses based on 16S rRNA and *gyrB*, respectively. The Roman numbers indicate the different clustering.](https://doi.org/10.1371/journal.pone.0186327.g001)
Polymorphism analysis of the internal transcribed spacer region

By PCR analysis of the ITS regions, 28 *B. coagulans* strains were grouped into 6 groups (Fig 2). The two bands at about 600 and 700 bp existed in the strains included in the major cluster of single locus strain typing of 16S rRNA and gyrB genes, except for the 6 strains listed above.

Strain typing using repetitive sequence-based PCR

Twenty-eight strains of *B. coagulans* were discriminated into 24 groups according to rep-PCR type by rep-PCR analysis (Fig 3). Three groups of rep-PCR type: rep-PCR type No. 2 (JCA1108, JCA1109, and JCA1115); rep-PCR type No. 12 (JCA1174 and JCA1175); and rep-PCR type No. 21 (DSM2350 and DSM2356) showed very high similarity (≥ 99%).

Microbiological analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

The 28 strains of *B. coagulans* were discriminated into 26 groups of MALDI type based on the similarity of MALDI-TOF MS patterns (Fig 4). The strains included in 2 groups of MALDI type, MALDI type No. 14 (JCA1174 and JCA1175), and MALDI type No. 23 (DSM2350 and DSM2356) were suggested to be identical by the analysis using SARAMIS software (Similarity: ≥ 80%). The 3 strains of rep-PCR type No. 2 (JCA1108, JCA1109, and JCA1115) were separated into different MALDI types (Fig 4, underlined).

Ribotyping

RiboPrinter system with EcoRI separated the 28 strains of *B. coagulans* into 23 ribogroups including four ribogroups: JCA1108, JCA1115, and JCA1116 (Ribogroup No. 2); JCA1121 and DSM2314 (Ribogroup No. 8); JCA1174 and JCA1175 (Ribogroup No. 12); DSM2350 and DSM2356 (Ribogroup No. 20) (Table 2) and 19 individual ribotypes (Table 2).

![Agarose gel electrophoresis patterns of PCR products showing the internal transcribed spacer (ITS) region in Bacillus coagulans strains. The alphabets indicated the each group which separated by the difference of ITS region.](https://doi.org/10.1371/journal.pone.0186327.g002)
Carbohydrate utilization of various *B. coagulans* strains

The 28 strains of *B. coagulans* possessed various types of carbohydrate utilization (Fig 5). The utilization of glycosides such as amygdalin, arbutin, esculin, salicin, and D-cellobiose was largely different between the strains. Although 4 strains, JCA1108, JCA1109, JCA1115, and
JCA1116, that exhibited strain similarity in the rep-PCR and/or Ribotyping analyses, also showed similar utilization of carbohydrates, the metabolic abilities of the glycosides were slightly different. On the other hand, JCA1174 and JCA1175 showed the same carbohydrate utilization. The same was true on DSM2350 and DSM2356. The strains JCA1174 and JCA1175...
could not utilize D-galactose and starch, whereas the strains DSM2350 and DSM2356 were able to utilize these sources. The latter two strains utilized D-sorbitol, but not D-raffinose.

### Discussion

The discriminatory power of strain typing of *B. coagulans* was compared among rep-PCR, MALDI-TOF MS, ribotyping, and carbohydrate utilization in the point of view of the precision and rapidity. Firstly, single gene sequencing analysis of the housekeeping genes 16S rDNA and gyrB provided only limited information for identification of species. It was difficult to distinguish *B. coagulans* strains by DNA sequencing of housekeeping genes and the PCR band patterns of ribosomal ITS region because of their small difference in DNA sequence. MLST might be useful for strain typing of *B. coagulans* because the genes that having higher evolution rates than housekeeping genes are usually chosen as the targets for MLST analysis [24]. MLST has been used as the standard method for a food-borne pathogen, *Bacillus cereus* [25]. MLST allows the characterization of the genetic diversity between environmental isolates using international databases and shows good reproducibility on the bacterial species with long-term epidemiological studies. However, it is difficult to apply to discriminate of bacteria

| Strain     | Ribogroup | rep-PCR type | MALDI type | Carbohydrate utilization | ITS type |
|------------|-----------|--------------|------------|--------------------------|----------|
| NBRC12583  | 1         | 1            | 1          | 1                        | a        |
| JCA1108    | 2         | 2            | 2          | 2                        | a        |
| JCA1109    | 3         | 2            | 3          | 3                        | a        |
| JCA1111    | 4         | 3            | 4          | 4                        | a        |
| JCA1115    | 2         | 2            | 5          | 5                        | a        |
| JCA1116    | 2         | 4            | 6          | 6                        | a        |
| JCA1117    | 5         | 5            | 7          | 7                        | b        |
| JCA1118    | 6         | 6            | 8          | 8                        | c        |
| JCA1120    | 7         | 7            | 9          | 9                        | d        |
| JCA1121    | 8         | 8            | 10         | 10                       | d        |
| JCA1122    | 9         | 9            | 11         | 11                       | c        |
| JCA1131    | 10        | 10           | 12         | 12                       | c        |
| JCA1158    | 11        | 11           | 13         | 13                       | e        |
| JCA1174    | 12        | 12           | 14         | 14                       | c        |
| JCA1175    | 12        | 12           | 14         | 14                       | c        |
| JCA1177    | 13        | 13           | 15         | 15                       | c        |
| JCA1178    | 14        | 14           | 16         | 16                       | c        |
| JCA1180    | 15        | 15           | 17         | 17                       | c        |
| JCA1182    | 16        | 16           | 18         | 18                       | c        |
| DSM2308    | 17        | 17           | 19         | 19                       | c        |
| DSM2311    | 18        | 18           | 20         | 20                       | f        |
| DSM2312    | 19        | 19           | 21         | 21                       | d        |
| DSM2314    | 8         | 20           | 22         | 22                       | d        |
| DSM2350    | 20        | 21           | 23         | 23                       | c        |
| DSM2356    | 20        | 21           | 23         | 23                       | c        |
| DSM2383    | 21        | 22           | 24         | 24                       | b        |
| DSM2384    | 22        | 23           | 25         | 25                       | c        |
| DSM2385    | 23        | 24           | 26         | 26                       | c        |

https://doi.org/10.1371/journal.pone.0186327.t002
including *B. coagulans* with poor genomic information in the databases and unidentified isolates from the environment. PFGE, the other gold standard of a strain typing method, needs the technical experience and is time-consuming. In this study, the rep-PCR and MALDI-TOF MS methods showed approximately equal performance in strain typing compared with Ribotyping, one of the most common and easy reference typing methods in the present circumstances [26]. Rep-PCR analysis targets repetitive sequences that are widely distributed in prokaryotic genomes. MALDI-TOF MS targets proteins with the low-molecular-weight ranges of \( m/z \) 3000 to 20000, especially ribosomal and envelope proteins. These analytical targets might contribute to the high discriminatory power of strain typing. We found that two groups, JCA1174 and JCA1175, and DSM2350 and DSM2356, showed high similarity by both rep-PCR and MALDI-TOF MS. In addition, carbohydrate utilization was fully consistent between DSM2350 and DSM2356 and between JCA1174 and JCA1175. The profile of carbohydrate utilization is one of the most important phenotype for the discrimination of bacterial strains [27, 28]. These results suggested that the bacterial protein profiles by MALDI-TOF MS are highly correlated with sequence-based strain typing methods as well as species identification. A recent study demonstrated the MALDI-TOF MS method was similar to MLST and superior to RAPD analyses in accuracy of strain typing of *Klebsiella pneumoniae*, which is an important pathogen in nosocomial infections [29]. In contrast, Berrazeg et al. reported that there was no direct correlation between MALDI-TOF MS-based typing and MLST for *K. pneumoniae* after consideration of different experimental conditions and analytical algorithms [30]. In our experimental conditions with different MALDI-TOF algorithms, the strain typing results of *B. coagulans*
were slightly different from our present study (data not shown). The appropriate analysis algorithm and cut-off settings of mass spectra are important for the development of strain typing by MALDI-TOF MS.

Strain typing using mass spectra by MALDI-TOF MS has already been proposed in the S10-GERMS (S10-spc-alpha operon gene encoded ribosomal protein mass spectrum) method [31–34]. The S10-GERMS method is focused on the S10-spc-alpha operon, where at least half of all ribosomal proteins are encoded. Some mass spectra can be correlated with theoretically calculated \( m/z \) ion peaks of ribosomal subunit proteins that are species- or strain-specific. This method is useful for accurate strain typing because of the quantitative correlation between the experimentally observed \( m/z \) values and theoretical values. However, it is difficult to apply sequence information of the S10-spc-alpha operon with unknown isolates or unidentified species from a standpoint of rapidity and versatility.

Our results suggested that the typing in combination of MALDI-TOF MS and rep-PCR analyses was effective for the strain typing of \( B. coagulans \). The target proteins of MALDI-TOF MS, including ribosomal and envelope proteins, and repetitive sequences targeted by rep-PCR in the bacterial genome were highly related. Consequently, it was possible to achieve precise discrimination between different strains of \( B. coagulans \). Further investigations on various strains with different characteristics among the same bacterial species are needed to demonstrate the usability and versatility of the MALDI-TOF MS-based method as a rapid bacterial strain typing method.

Acknowledgments

We would like to give thanks to Mr. Shima, SHIMADZU CO. Ltd., Japan, who provided carefully considered feedback and valuable comments about MALDI-TOF MS analysis.

Author Contributions

Conceptualization: Jun Sato, Motokazu Nakayama, Takumi Sonoda, Takahisa Miyamoto.

Data curation: Jun Sato, Ayumi Tomita.

Investigation: Jun Sato, Ayumi Tomita.

Project administration: Takumi Sonoda, Motomitsu Hasumi.

Supervision: Motokazu Nakayama, Motomitsu Hasumi.

Visualization: Ayumi Tomita.

Writing – original draft: Jun Sato.

Writing – review & editing: Jun Sato, Takahisa Miyamoto.

References

1. Wilson KH, Biltongton RB, Greene RC. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. Journal of clinical microbiology. 1990; 28(9):1942–6. Epub 1990/09/01. PMID: 2095137; PubMed Central PMCID: PMC268083.

2. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95(6):3140–5. Epub 1998/04/18. PMID: 9501229; PubMed Central PMCID: PMC19708.

3. Liu PY, Ke SC, Chen SL. Use of pulsed-field gel electrophoresis to investigate a pseudo-outbreak of Bacillus cereus in a pediatric unit. Journal of clinical microbiology. 1997; 35(6):1533–5. Epub 1997/06/01. PMID: 9163476; PubMed Central PMCID: PMC229781.
4. Thorsen L, Hansen BM, Nielsen KF, Hendriksen NB, Phipps RK, Budde BB. Characterization of emetic Bacillus weihenstephanensis, a new cereulide-producing bacterium. Applied and environmental microbiology. 2006; 72(7):5118–21. Epub 2006/07/06. https://doi.org/10.1128/AEM.01700-06 PMID: 16820519; PubMed Central PMCID: PMC1489381.

5. Hill KK, Ticknor LO, Okinaka RT, Asay M, Blair H, Bliss KA, et al. Fluorescent amplified fragment length polymorphism analysis of Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis isolates. Applied and environmental microbiology. 2004; 70(2):1068–80. Epub 2004/02/10. https://doi.org/10.1128/AEM.70.2.1068-1080.2004 PMID: 14766590; PubMed Central PMCID: PMC348840.

6. Shangkuan YH, Yang JF, Lin HC, Shaio MF. Comparison of PCR-RFLP, ribotyping and ERIC-PCR for typing Bacillus anthracis and Bacillus cereus strains. Journal of applied microbiology. 2000; 89(3):452–62. Epub 2000/10/06. PMID: 11021577.

7. MacCannell D. Bacterial strain typing. Clinics in laboratory medicine. 2013; 33(3):629–50. Epub 2013/08/13. https://doi.org/10.1016/j.cll.2013.03.005 PMID: 23931842.

8. Deschaght P, Van Sinaey L, Decat E, Van Mechelen E, Brisse S, Vaneechoutte M. Rapid genotyping of Achromobacter xylosoxidans, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Stenotrophomonas maltophilia isolates using melting curve analysis of RAPD-generated DNA fragments (McRAPD). Research in microbiology. 2011; 162(4):386–92. Epub 2011/02/16. https://doi.org/10.1111/j.1462-2920.2010.01856.x PMID: 21320595.

9. Ishii S, Sadowsky MJ. Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity and ecology. Environmental microbiology. 2003; 11(4):733–40. Epub 2003/02/12. https://doi.org/10.1111/j.1462-2920.2002.00799.x PMID: 12340926.

10. Serwecinska L, Ciesliskowski T, Pytlos M, Jaworski A, Kaca W. Genomic fingerprinting of Proteus species using repetitive sequence based PCR (rep-PCR). Acta microbiologica Polonica. 1998; 47(3):313–9. Epub 1999/02/17. PMID: 9993014.

11. Tenover FC, Gay EA, Frye S, Eills SJ, Healy M, McGowan JE Jr. Comparison of typing results obtained for methicillin-resistant Staphylococcus aureus isolates with the DiversiLab system and pulsed-field gel electrophoresis. Journal of clinical microbiology. 2003; 41(6):2111–17. Epub 2003/05/03. https://doi.org/10.1128/JCM.41.6.2111-2117.2003 PMID: 12844337.

12. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and environmental microbiology. 2008; 74(17):5402–7. Epub 2008/07/09. https://doi.org/10.1128/AEM.00268-07 PMID: 18606788; PubMed Central PMCID: PMC2755641.

13. Stephane R, Cernela N, Ziegler D, Pfluger V, Tonolla M, Ravasi D, et al. Rapid species specific identification and subtyping of Yersinia enterocolitica by MALDI-TOF mass spectrometry. Journal of microbiological methods. 2011; 87(2):150–3. Epub 2011/09/15. https://doi.org/10.1016/j.mimet.2011.08.016 PMID: 21914454.

14. Fernandez-No IC, Bohme K, Diaz-Bao M, Cepeda A, Barros-Velazquez J, Calo-Mata P. Characterisation and profiling of Bacillus subtilis, Bacillus cereus and Bacillus licheniformis by MALDI-TOF mass fingerprinting. Food microbiology. 2013; 33(2):235–42. Epub 2012/12/04. https://doi.org/10.1016/j.fm.2012.09.022 PMID: 23200657.

15. Schoeni JL, Wong AC. Bacillus cereus food poisoning and its toxins. Journal of food protection. 2005; 68(3):636–48. Epub 2005/03/18. PMID: 15771199.

16. Marten P, Smalla K, Berg G. Genotypic and phenotypic differentiation of an antifungal biocontrol strain belonging to Bacillus subtilis. Journal of applied microbiology. 2000; 89(3):463–71. Epub 2000/10/06. PMID: 11021578.

17. Palop A, Raso J, Pagan R, Condon S, Sala FJ. Influence of pH on heat resistance of spores of Bacillus coagulans in buffer and homogenized foods. International journal of food microbiology. 1999; 46 (3):243–9. Epub 1999/04/01. PMID: 10109094.

18. Cosentino S, Mulargia AF, Pisano B, Tuveri P, Palmas F. Incidence and biochemical characteristics of Bacillus flora in Sardinian dairy products. International journal of food microbiology. 1997; 38(2–3):243–8. Epub 1998/03/20. PMID: 9506289.

19. Hong HA, Duc le H, Cutting SM. The use of bacterial spore formers as probiotics. FEMS microbiology reviews. 2005; 29(4):813–35. Epub 2005/08/17. https://doi.org/10.1016/j.femsre.2004.12.001 PMID: 16102604.

20. Allerberger F, Fritschel SJ. Use of automated ribotyping of Austrian Listeria monocytogenes isolates to support epidemiological typing. Journal of microbiological methods. 1999; 35(3):237–44. Epub 1999/05/20. PMID: 10333075.

21. Wang LT, Lee FL, Tai CJ, Kasi H. Comparison of gyrB gene sequences, 16S RNA gene sequences and DNA-DNA hybridization in the Bacillus subtilis group. International journal of systematic and
22. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution. 1987; 4(4):406–25. Epub 1987/07/01. PMID: 3447015.

23. Daffonchio D, Clerf A, Brusetti L, Rizzi A, Mora D, Boudabous A, et al. Nature of polymorphisms in 16S-23S rRNA gene intergenic transcribed spacer fingerprinting of Bacillus and related genera. Applied and environmental microbiology. 2003; 69(9):5128–37. Epub 2003/09/06. https://doi.org/10.1128/AEM.69.9.5128-5137.2003 PMID: 12957895; PubMed Central PMCID: PMC194986.

24. Helgason E, Tourasse NJ, Meisal R, Caugant DA, Kolsto AB. Multilocus sequence typing scheme for bacteria of the Bacillus cereus group. Applied and environmental microbiology. 2004; 70(1):191–201. Epub 2004/01/09. https://doi.org/10.1128/AEM.70.1.191-201.2004 PMID: 14711642; PubMed Central PMCID: PMC321270.

25. Priest FG, Barker M, Baillie LW, Holmes EC, Maiden MC. Population structure and evolution of the Bacillus cereus group. Journal of bacteriology. 2004; 186(23):7959–70. Epub 2004/11/18. https://doi.org/10.1128/JB.186.23.7959-7970.2004 PMID: 15547268; PubMed Central PMCID: PMC529064.

26. Thompson FL, Iida T, Swings J. Biodiversity of vibrios. Microbiology and molecular biology reviews: MMBR. 2004; 68(3):403–31, table of contents. Epub 2004/09/09. https://doi.org/10.1128/MMBR.68.3.403-431.2004 PMID: 15353563; PubMed Central PMCID: PMC515257.

27. Nigatu A. Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact. sake, Lact. parabuchneri, Lact. gallinarum, Lact. casei, Weissella minor and related taxa isolated from kocho and tef. Journal of applied microbiology. 2000; 89(6):969–78. Epub 2000/12/21. PMID: 11123470.

28. Pinto B, Chenoll E, Aznar R. Identification and typing of food-borne Staphylococcus aureus by PCR-based techniques. Systematic and applied microbiology. 2005; 28(4):340–52. Epub 2005/07/07. https://doi.org/10.1016/j.syapm.2005.01.002 PMID: 15997707.

29. Sachse S, Bresan S, Erhard M, Edel B, Pfister W, Saupe A, et al. Comparison of multilocus sequence typing, RAPD, and MALDI-TOF mass spectrometry for typing of beta-lactam-resistant Klebsiella pneumoniae strains. Diagnostic microbiology and infectious disease. 2014; 80(4):267–71. Epub 2014/10/01. https://doi.org/10.1016/j.diagmicrobio.2014.09.005 PMID: 25266674.

30. Berrezeg M, Diene SM, Drissi M, Kempf M, Richet H, Landraud L, et al. Biotyping of multidrug-resistant Klebsiella pneumoniae clinical isolates from France and Algeria using MALDI-TOF MS. PloS one. 2013; 8(4):e61428. Epub 2013/04/27. https://doi.org/10.1371/journal.pone.0061428 PMID: 23620754; PubMed Central PMCID: PMC3631213.

31. Hotta Y, Sato J, Sato H, Hosoda A, Tamura H. Classification of the genus Bacillus based on MALDI-TOF MS analysis of ribosomal proteins coded in S10 and spc operons. Journal of agricultural and food chemistry. 2011; 59(10):5222–30. Epub 2011/04/08. https://doi.org/10.1021/jf2004095 PMID: 21469741.

32. Sato H, Torimura M, Kitahara M, Ohkuma M, Hotta Y, Tamura H. Characterization of the Lactobacillus casei group based on the profiling of ribosomal proteins coded in S10-spc-alpha operons as observed by MALDI-TOF MS. Systematic and applied microbiology. 2012; 35(7):447–54. Epub 2012/10/27. https://doi.org/10.1016/j.syapm.2012.08.008 PMID: 23099260.

33. Ojima-Kato T, Yamamoto N, Suzuki M, Fukunaga T, Tamura H. Discrimination of Escherichia coli O157, O26 and O111 from other serovars by MALDI-TOF MS based on the S10-GERMS method. PloS one. 2014; 9(11):e113458. Epub 2014/11/21. https://doi.org/10.1371/journal.pone.0113458 PMID: 25411793; PubMed Central PMCID: PMC4239071.

34. Ojima-Kato T, Yamamoto N, Takahashi H, Tamura H. Matrix-assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Can Precisely Discriminate the Lineages of Listeria monocytogenes and Species of Listeria. PloS one. 2016; 11(7):e0159730. Epub 2016/07/22. https://doi.org/10.1371/journal.pone.0159730 PMID: 27442502.