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Identification of *Fusobacterium* Species Using Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry by Updating ASTA CoreDB

Shin Young Yun[1], Yunhee Lee[1], Juwon Hong[2], Dong-Chan Kim[2], Hyukmin Lee[1], Dongeun Yong[1], Yun Kyong Lim[3], Joong-Ki Kook[3], and Kyungwon Lee[1,4]

[1]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul;
[2]R&D Center, NOSQUEST Inc., Yongin;
[3]Korean Collection for Oral Microbiology and Department of Oral Biochemistry, School of Dentistry, Chosun University, Gwangju;
[4]Seoul Clinical Laboratories Academy, Yongin, Korea.

Purpose: *Fusobacterium* species can cause infections, and associations with cancer are being increasingly reported. As their clinical significance differs, accurate identification of individual species is important. However, matrix-assisted laser desorption/ionization-time of flight mass spectrometry has not been found to be effective in identifying *Fusobacterium* species in previous studies. In this study, we aimed to improve the accuracy and efficacy of identifying *Fusobacterium* species in clinical laboratories.
Materials and Methods: In total, 229 *Fusobacterium* isolates were included in this study. All isolates were identified at the species level based on nucleotide sequences of the 16S ribosomal RNA gene and/or DNA-dependent RNA polymerase β-subunit gene (*rpoB*). Where necessary, isolates were identified based on whole genome sequences. Among them, 47 isolates were used for updating the ASTA database, and 182 isolates were used for the validation of *Fusobacterium* spp. identification.
Results: *Fusobacterium* isolates used for validation (182/182) were correctly identified at the genus level, and most (180/182) were correctly identified at the species level using the ASTA MicroIDSys system. Most of the *F. nucleatum* isolates (74/75) were correctly identified at the subspecies level.
Conclusion: The updated ASTA MicroIDSys system can identify nine species of *Fusobacterium* and four subspecies of *F. nucleatum* in good agreement. This tool can be routinely used in clinical microbiology laboratories to identify *Fusobacterium* species and serve as a springboard for future research.

Key Words: *Fusobacterium*, *Fusobacterium nucleatum*, *Fusobacterium necrophorum*, spectrometry, mass, matrix-assisted laser desorption-ionization

INTRODUCTION

Fusobacterium spp. are gram negative, rod shaped, obligate anaerobic bacteria and are difficult to culture and require long incubation times.[1-3] Generally, *Fusobacterium* spp. act as the normal flora of the female genital tract, gastrointestinal tract, and oral cavity. They can, however, cause infections, and their association with cancer is being increasingly reported.[4-7] *Fusobacterium* necrophorum can cause acute tonsillitis, peritonsillar abscess[4,6,8] and Lemierre's disease, accompanied by internal jugular thrombophlebitis and septic emboli.[9] In addition, *Fusobacterium* nucleatum might be associated with the development of colorectal cancer, and a high abundance of *F. nucleatum* in colorectal tumors has been shown to be associ-

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Co-corresponding authors: Hyukmin Lee, MD, PhD, Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
E-mail: hmlee71@yuhs.ac and
Joong-Ki Kook, DDS, PhD, Korean Collection for Oral Microbiology and Department of Oral Biochemistry, School of Dentistry, Chosun University, 7 Chosundae-gil, Dong-gu, Gwangju 61452, Korea.
E-mail: jkkook@chosun.ac.kr

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ated with poor overall survival.[10,11] Other studies have reported molecular features of *F. nucleatum* to be associated with colorectal carcinogenesis.[12,13] *F. nucleatum* is classified into four subspecies: *animalis*, *nucleatum*, *polymorphum*, and *vincentii*, and each subspecies might have different pathogenicity.[14-16] These species show different biofilm forming ability in vitro,[17,18] and associations with colorectal cancer at the subspecies level are being studied.[19] *F. nucleatum* subsp. *animalis* may also be associated with inflammatory bowel disease,[20] and *F. nucleatum* subsp. *nucleatum* is frequently isolated in periodontal disease.[14] Other relatively rare species, including *Fusobacterium gonidiaformans*, *Fusobacterium hwasookii*, and *Fusobacterium mortiferum*, have been isolated from infections at other sites.[21-23] Recently, researchers have suggested that non-nucleatum *Fusobacterium* may be associated with colorectal cancer.[24] Therefore, easy and accurate identification of these organisms at the subspecies and species levels is important in clinical laboratories.

Recently, whole genome sequence comparison methods, such as genome-to-genome distance or average nucleotide identity analysis, and 16S ribosomal RNA gene (16S rDNA) nucleotide comparison methods have been used as gold standards for the classification of bacteria at the species or subspecies level.[25,26] Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) is presently the most widely used tool for identifying microorganisms in clinical microbiology laboratories. The accuracy of identification using MALDI-TOF MS depends on the quality of its database. However, Bruker Biotyper (Bruker Daltonics, Bremen, Germany) includes ten *Fusobacterium* species, and VITEK MS (bioMérieux, Marcy l'Etoile, France), which is also one of the most widely used MALDI-TOF systems, includes only four *Fusobacterium* species. As such, both MALDI-TOF MS methods have been found to be insufficient in identifying variable *Fusobacterium* species in previous studies.[27-31] In one study using the Bruker Biotyper, only 20% (1/5) of *Fusobacterium* species were identified at the genus level, and 75% (3/4) of *F. nucleatum* isolates were not identified.[31] In another study using the Bruker Biotyper, only 25% (3/12) of *Fusobacterium* species were identified at the species level, with a score above 1.8, and 16.7% (2/12) were not correctly identified at both the genus and species level.[30] A previous study of *F. nucleatum* using VITEK MS showed correct identification of 82.4% (28/34) of isolates at the subspecies level.[28]

The ASTA MicroIDSys system (ASTA Inc., Suwon, South Korea) is a MALDI-TOF MS system developed in 2014. The database includes only six *Fusobacterium* species: *Fusobacterium canifelinum*, *F. mortiferum*, *F. necrophorum*, *F. nucleatum*, *Fusobacterium ulcerans*, and *Fusobacterium varium*. In addition, the database is not able to differentiate between the four subspecies of *F. nucleatum*. In this study, to improve the accuracy and efficacy of identifying *Fusobacterium* species in clinical laboratories, we updated the ASTA database using seven *Fusobacterium* species (total 47 isolates) and four *F. nucleatum* subspecies (total 25 isolates) (Table 1). In addition, we evaluated the function of the ASTA MicroIDSys system in identifying *Fusobacterium* isolates at the species or subspecies level.

MATERIALS AND METHODS

Bacterial isolates

A total of 229 *Fusobacterium* isolates belonged to nine species and four subspecies of *F. nucleatum* (Table 1). Of these, 171 were collected at Yonsei University Severance Hospital in Seoul from January 2006 to January 2021, and 53 were obtained from Korean Collection for Oral Microbiology of Chosun University in Gwangju, Korea. Five isolates of ATCC, one *F. nucleatum* subsp. *animalis* (ATCC 51191), two *F. nucleatum* subsp. *nucleatum* (ATCC 25586 and ATCC23726), one *F. nucleatum* subsp. *polymorphum* (ATCC10953), and one *F. nucleatum* subsp. *vincentii* (ATCC49256) were also included. The collected isolates were stored in skimmed milk at -80°C before analysis. The selection of isolates to be used for validation and database upgrade were randomly determined. During the confirmation of identification by 16S rDNA sequencing, *F. mortiferum* and *F. necrophorum* seemed to be well identified empirically; therefore, we only conducted validation.

Molecular identification method

All clinical isolates from Severance Hospital were identified at the species level by sequencing of 16S rDNA, and *rpoB* genes were also analyzed for subspecies identification of *F. nucleatum*. A k-mer based method for the identification of all *F. gonidiaformans*, *F. hwasookii*, *Fusobacterium periodonticum*

Table 1. Number of Species used for Database Upgrade and Analysis

Species	The number of isolates		
	Total	Used for database upgrade	Used for validation
F. gonidiaformans	3	1	2
F. hwasookii	6	3	3
F. mortiferum	14	0	14
F. necrophorum	25	0	25
F. nucleatum	100	25	75
F. nucleatum subsp. animalis	31	7	24
F. nucleatum subsp. nucleatum	19	6	13
F. nucleatum subsp. polymorphum	31	7	24
F. nucleatum subsp. vincentii	19	5	14
F. periodonticum	11	3	8
F. pseudoperiodonticum	6	5	1
F. ulcerans	11	5	6
F. varium	53	5	48
Total	229	47	182

No strain was used for both upgrading and validation simultaneously.

and *Fusobacterium pseudoperiodonticum* isolates was performed using whole genome sequencing.[32] In addition, it was performed on some *F. nucleatum* isolates that showed poor 16S rDNA and *rpoB* gene sequencing results. All strains from Korean Collection for Oral Microbiology (KCOM, Gwangju, Korea) were isolated from the oral cavities of a Korean population and identified at the species level based on whole-genome sequences.[22,33]

The generation of a local database–combined mass spectrum (CMS) creation

To generate a local database, 47 *Fusobacterium* isolates were used for CMS creation. A bacterial isolate colony was directly placed on the MALDI target plate and allowed to dry, after which 1.5 μL of 70% formic acid was applied to the sample and air dried. The matrix solution (1.5 μL) was composed of 10 g/L α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich, St. Louis, MO, USA) saturated in 2.5% trifluoroacetic acid (Sigma-Aldrich) and 50% acetonitrile (Merck, Darmstadt, Germany). Each isolate yielded 20 spectra. According to ASTA's recommendations, a single CMS was created using 20 isolated spectra. Each spectrum was created using 1200 laser shots and analyzed using a mass m/z of 2000–20000. The ASTA MicroID Standard (AMS) was used for the calibration (ASTA Inc., Suwon, Korea). Previously, six *Fusobacterium* sp. species with 68 CMS have been used to identify clinical isolates. Following this update, 11 *Fusobacterium* spp. and 117 CMS were used. A new version of the ASTA CoreDB was created using the newly updated CMS (ver.1.27.04).

Four and six CMS were obtained to evaluate the performance of differentiating between *F. periodonticum* and *F. pseudoperiodonticum*, respectively. The resulting spectrum data were composed of mass bins of relative intensity (%) (Supplementary Fig. 1, only online). We could compare the percentages of intensity signals and mass-to-charge ratios (m/z) of protein components by binning of the spectra. To visualize the difference more clearly, a heat map of cross-matched data was created, as well as principal component analysis (PCA) (Supplementary Fig. 1B and C, only online). The distance in the heat map was based on the Spearman correlation value. Closely related spectra are marked in hot colors, and the unrelated spectra are indicated by cold colors. In the PCA plot, species CMS are described by two vectors, the first principal component (Dim1) and the other crossing it at right angles (Dim2). The domain of each species is illustrated by a colored ellipse (confidence level 99.9%).

Validation of the upgraded ASTA MicroIDSys database

After upgrading the database, the remaining 182 isolates were used for validation of the ASTA MicroIDSys database according to the manufacturer's instructions, which was prescribed in the CMS creation method. Target samples were analyzed using MicroIDSys and control software (ver. 3.1.4). The acceptable criteria for the identification score was 140 or more, which is identical to the manufacturer's recommendations. Each isolate was tested in duplicate. There was a total of 27 cases in which repeated tests were inconsistent. Twenty-five of them were "un-identified" in the first of the two tests, with accurate results coming from the second. All cases of un-identified results were caused by poor-quality spectra, and the most likely reason for this is that the target spot preparation was suboptimal: either too much or too little organism was applied or matrix solution was not added.[34] The remaining two cases were identified accurately in one test, but with different species in the other test. These two cases were considered to be incorrectly identified in consideration of the possibility of inaccurate results.

RESULTS

All *Fusobacterium* species (100%, 182/182) were correctly identified at the genus level, and most (98.90%, 180/182) were correctly identified by the ASTA MicroIDSys system at the species level (Table 2). *F. gonidiaformans* (100%, 2/2), *F. hwasookii* (100%, 3/3), *F. mortiferum* (100%, 14/14), *F. necrophorum* (100%, 25/25), *F. pseudoperiodonticum* (100%, 1/1), *F. ulcerans* (100%, 6/6), and *F. varium* (100%, 48/48) were correctly identified at the species level.

Most of the *F. nucleatum* isolates (98.67%, 74/75) were correctly identified at the subspecies level. *F. nucleatum* subsp. *nucleatum* (100%, 13/13), *F. nucleatum* subsp. *polymorphum* (100%, 24/24), and *F. nucleatum* subsp. *vincentii* (100%, 14/14) were correctly identified at the subspecies level. Of the 24 *F. nucleatum* subsp. *animalis* isolates, 23 were correctly identified at the subspecies level (95.83%, 23/24), and one isolate was identified as *F. canifelinum*.

Although *F. periodonticum* and *F. pseudoperiodonticum* were difficult to differentiate using 16S rDNA and *rpoB* sequencing,[33] the protein spectra of *F. periodonticum* and *F. pseudoperiodonticum* showed sharp differences (Supplementary Fig. 1, only online). In the heat map, distance values were derived by subtracting the Spearman correlation value from one and were 0.0–0.6 among *F. periodonticum*, 0.0–0.8 among *F. pseudoperiodonticum*, and 0.5–1.0 between F. *periodonticum* and *F. pseudoperiodonticum* (Supplementary Fig. 1B, only online). In the PCA plot, two species could be divided clearly by the first principal component. Unfortunately, the first principal component had more than hundreds of factors contributing in various proportions, and thus, we could not derive a single protein element that separated the two species (Supplementary Fig. 1C, only online). Finally, ASTA MicroIDSys could correctly identified *F. periodonticum* and *F. pseudoperiodonticum* (88.89%, 8/9) at the species level (Table 2). Only one *F. periodonticum* isolate was identified as *F. pseudoperiodonticum*.

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Table 2. Comparison of Identification Results of the 182 *Fusobacterium* Isolates by ASTA MicroIDSys and Reference Method

Species (no. isolates)	Correct identification to			% agreement at species level*
	Genus level	Species level	Subspecies level*	
F. gonidiaformans (2)	0	2	-	100
F. hwasookii (3)	0	3	-	100
F. mortiferum (14)	0	14	-	100
F. necrophorum (25)	0	25	-	100
F. nucleatum (75)	1	74	-	98.67
*F. nucleatum subsp. animalis** (24)	1	0	23*	95.83*
*F. nucleatum subsp. nucleatum** (13)	0	0	13*	100*
*F. nucleatum subsp. polymorphum** (24)	0	0	24*	100*
*F. nucleatum subsp. vincentii** (14)	0	0	14*	100*
F. periodonticum (8)	1	7	-	87.5
F. pseudoperiodonticum (1)	0	1	-	100
F. ulcerans (6)	0	6	-	100
F. varium (48)	0	48	-	100
Total (182)	2	180	-	98.90

Reference methods consisted of 16S rDNA sequencing for species identification, *rpoB* gene sequencing for subspecies identification in *F. nucleatum*, and whole genome sequencing.
*The identification to the subspecies level was indicated only for *F. nucleatum*. In this cases, %agreement was calculated at the subspecies level.

DISCUSSION

Fusobacterium nucleatum shows genetic variability, has four subspecies, and seems to be reclassified to the species level.[35] As such it is important to identify *Fusobacterium* species correctly, especially at the subspecies level for *F. nucleatum*. MALDI-TOF MS is easy to use, inexpensive, and requires less than a minute for microorganism identification.[31,36,37] However, the quality of the supporting database can influence on the performance of the identification, and well-defined microorganisms are required for improvement of the database. To date, frequently isolated species could be identified with the ASTA MicroIDSys, but relatively rare species could not be identified. To solve this problem, we collected as many *Fusobacterium* species from clinical samples as possible and upgraded the ASTA MicroIDSys database. *F. canifelinum*, *F. mortiferum*, *F. necrophorum*, *F. nucleatum*, *F. ulcerans*, and *F. varium* were identified using the ASTA database. However, *F. ulcerans* and *F. varium* were sometimes not correctly identified as others, and thus, these species were also included in the updated list. All isolates in this study were confirmed by molecular testing, 16S rDNA sequencing, *rpoB* sequencing, and whole genome sequencing. All *F. gonidiaformans*, *F. hwasookii*, *F. periodonticum*, and *F. pseudoperiodonticum* isolates were confirmed by whole genome sequencing.

Molecular methods are more accurate for bacterial identification than MALDI-TOF MS, but they are time-consuming and expensive; therefore, laboratories cannot use them for routine bacterial identification methods. Finally, we developed and validated an ASTA database to identify *Fusobacterium* species in clinical laboratories. 16S rDNA and *rpoB* are well-conserved and useful regions for bacterial identification, but some species show nearly 100% similarity of these regions. Even in those cases, proteomic analysis like MALDI-TOF MS can be a useful identification method, because expressed proteins are coded by various genes.

Two isolates were identified to the genus level. One isolate of *F. nucleatum* subsp. *animalis* was identified as *F. canifelinum* and one isolate of *F. periodonticum* was identified as *F. pseudoperiodonticum*. All the isolates were correctly identified using repeated tests with ASTA MicroIDSys. These discrepancies may be caused by the similarity of the spectra, for which adding more spectral information may be helpful. The other isolates of the same species were correctly identified.

F. periodonticum and *F. pseudoperiodonticum* are difficult to distinguish in clinical laboratories. The distinction between the two species was described by mass binning, a heat map, and PCA plotting. Interestingly, nearly all *F. periodonticum* and *F. pseudoperiodonticum* were correctly identified at the species level using the ASTA MicroIDSys. Overall, same species were more closely related than the other species. In a PCA plot, the two species could be divided clearly by the first principal component. Each ellipse contains 99.9% of all samples drawn under Gaussian distribution. Thus, we could see the differences in the protein spectra of the two species, and these differences seemed to allow ASTA MicroIDSys to differentiate between the two species.

The ASTA MALDI-TOF database is based on spectra obtained by analyzing various isolates received through official procedures in large hospitals and institutions in Korea, as well as clinical isolates from foreign authorized institutions. ASTA's protein spectrum database is constructed by collecting 20 to 30 spectra per isolate for each isolate. Only spectra that pass verification, checking the relationship with the spectra of the

existing database, are added to the new database. Therefore, the accuracy of microbial identification is very high.[38,39] In addition, it has a faster laser speed of >1000 Hz, compared to other equipment, and the plate loading time is shorter than 1.5 minutes. It can identify 96 isolates in about 20 minutes. Also, the sample preparation kit developed by the company is also provided to the users, making it is possible to maintain uniformity of various conditions until the sample spectrum is obtained. Lastly, based on the artificial intelligence machine learning data processing process developed by the ASTA software team, a self-developed database is established that can accurately distinguish isolates at not only the genus and species level but also the subspecies level. Recently, simple microbial identification and a database that can distinguish between resistant and susceptible isolates for specific antibiotics are being developed.

A limitation of this study was that rare species of *Fusobacterium* were not included in the updated list, such as *F. canifelinum*, *F. equinum*, and *F. gastrosuis*. Secondly, we were unable to collect clinical isolates of *F. canifelinum*. Thirdly, the identification data of previous database was not available, and we could not compare the data before and after upgrading database. Finally, the numbers of some species were too small. For example, only one isolate of *F. pseudoperiodonticum* was used for validation, and further investigation using additional isolates is needed.

However, ASTA MicroIDsys could identify nine species of *Fusobacterium* and four subspecies of *F. nucleatum*, which may be associated with colorectal cancer, with good agreement after upgrading the ASTA MicroIDSys database. A previous report on *F. nucleatum* subspecies identification by MALDI-TOF MS showed only 82.4% (28/34) of isolates were correctly identified at the subspecies level 28, and a better identification ability (98.67%) was shown in this study. In addition, *F. periodonticum* and *F. pseudoperiodonticum* are known to be differentiated only by the analysis of whole genome sequences, but we confirmed differences in their protein spectra and proved the identification ability of ASTA MicroIDSys. This allows clinical laboratories to identify them more easily and rapidly.

In conclusion, the updated ASTA MicroIDSys can identify *Fusobacterium* species in good agreement. Therefore, this tool can be routinely used in clinical microbiology laboratories to identify *Fusobacterium* species, and it can serve as a springboard for future research including *F. nucleatum* and colorectal cancer.

AUTHOR CONTRIBUTIONS

Conceptualization: Kyungwon Lee. Data curation: Joong-Ki Kook, Yun Kyong Lim, and Hyukmin Lee. Formal analysis: Joong-Ki Kook, Yun Kyong Lim, and Hyukmin Lee. Investigation: Yunhee Lee. Methodology: Kyungwon Lee and Hyukmin Lee. Project administration: Kyungwon Lee, Hyukmin Lee, and Joong-Ki Kook. Resources: Hyukmin Lee. Software: Dong-Chan Kim and Juwon Hong. Supervision: Kyungwon Lee, Hyukmin Lee, and Joong-Ki Kook. Validation: Yunhee Lee. Visualization: Shin Young Yun. Writing—original draft: Shin Young Yun. Writing—review & editing: Kyungwon Lee, Hyukmin Lee, Dong-Chan Kim, Juwon Hong, Joong-Ki Kook, and Dongeun Yong. Approval of final manuscript: all authors.

ORCID iDs

Shin Young Yun https://orcid.org/0000-0003-1020-7147
Yunhee Lee https://orcid.org/0000-0002-3728-791X
Juwon Hong https://orcid.org/0000-0001-8771-6463
Dong-Chan Kim https://orcid.org/0000-0002-9361-8023
Hyukmin Lee https://orcid.org/0000-0002-8523-4126
Dongeun Yong https://orcid.org/0000-0002-1225-8477
Yun Kyong Lim https://orcid.org/0000-0002-6215-8204
Joong-Ki Kook https://orcid.org/0000-0003-2628-2870
Kyungwon Lee https://orcid.org/0000-0003-3788-2134

REFERENCES

1. Langworth BF. Fusobacterium necrophorum: its characteristics and role as an animal pathogen. Bacteriol Rev 1977;41:373-90.
2. Spaulding EH, Rettger LF. The Fusobacterium genus: II. Some observations on growth requirements and variation. J Bacteriol 1937;34:549-63.
3. Slots J, Potts TV, Mashimo PA. Fusobacterium periodonticum, a new species from the human oral cavity. J Dent Res 1983;62:960-3.
4. Bank S, Jensen A, Nielsen HM, Kristensen LH, Voldstedlund M, Prag J. Fusobacterium necrophorum findings in Denmark from 2010 to 2014 using data from the Danish microbiology database. APMIS 2016;124:1087-92.
5. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 2015;23:141-7.
6. Holm K, Bank S, Nielsen H, Kristensen LH, Prag J, Jensen A. The role of Fusobacterium necrophorum in pharyngotonsillitis-A review. Anaerobe 2016;42:89-97.
7. Huggan PJ, Murdoch DR. Fusobacterial infections: clinical spectrum and incidence of invasive disease. J Infect 2008;57:283-9.
8. Ehlers Klug T, Rusan M, Fuursted K, Ovesen T. Fusobacterium necrophorum: most prevalent pathogen in peritonsillar abscess in Denmark. Clin Infect Dis 2009;49:1467-72.
9. Johannesen K, Bødtger U, Heltberg O. Lemierre's syndrome: the forgotten disease. J Thromb Thrombolysis 2014;37:246-8.
10. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe 2013;14:195-206.
11. Gethings-Behncke C, Coleman HG, Jordao HWT, Longley DB, Crawford N, Murray LJ, et al. Fusobacterium nucleatum in the colorectum and its association with cancer risk and survival: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2020;29:539-48.
12. Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. Gut 2016;65:1973-80.
13. Tahara T, Yamamoto E, Suzuki H, Maruyama R, Chung W, Garriga J, et al. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. Cancer Res 2014;74:1311-8.
14. Genco RJ, Loos BG. The use of genomic DNA fingerprinting in studies of the epidemiology of bacteria in periodontitis. J Clin Periodontol 1991;18:396-405.
15. Gharbia SE, Shah HN, Lawson PA, Haapasalo M. Distribution and frequency of Fusobacterium nucleatum subspecies in the human oral cavity. Oral Microbiol Immunol 1990;5:324-7.

https://doi.org/10.3349/ymj.2022.0271

16. Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res 2013;92:485-91.
17. Muchova M, Balacco DL, Grant MM, Chapple ILC, Kuehne SA, Hirschfeld J. Fusobacterium nucleatum subspecies differ in biofilm forming ability in vitro. Front Oral Health 2022;3:853618.
18. Thurnheer T, Karygianni L, Flury M, Belibasakis GN. Fusobacterium species and subspecies differentially affect the composition and architecture of supra- and subgingival biofilms models. Front Microbiol 2019;10:1716.
19. Bi D, Zhu Y, Gao Y, Li H, Zhu X, Wei R, et al. A newly developed PCR-based method revealed distinct Fusobacterium nucleatum subspecies infection patterns in colorectal cancer. Microb Biotechnol 2021;14:2176-86.
20. Han YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. J Clin Microbiol 2009;47:38-47.
21. Vidaurrazaga MM, McKinley GF, Camins BC. Postpartum Fusobacterium gonidiaformans bacteremia. Anaerobe 2020;62:102168.
22. Cho E, Park SN, Lim YK, Shin Y, Paek J, Hwang CH, et al. Fusobacterium hwasookii sp. nov., isolated from a human periodontitis lesion. Curr Microbiol 2015;70:169-75.
23. Stavreas NP, Amanatidou CD, Hatzimanolis EG, Legakis I, Naoum G, Lakka-Papadodima E, et al. Thyroid abscess due to a mixed anaerobic infection with Fusobacterium mortiferum. J Clin Microbiol 2005;43:6202-4.
24. He Y, Mujagond P, Tang W, Wu W, Zheng H, Chen X, et al. Non-nucleatum Fusobacterium species are dominant in the Southern Chinese population with distinctive correlations to host diseases compared with F. nucleatum. Gut 2021;70:810-2.
25. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 2018;68:461-6.
26. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 2009;106:19126-31.
27. Veloo AC, Welling GW, Degener JE. The identification of anaerobic bacteria using MALDI-TOF MS. Anaerobe 2011;17:211-2.
28. Nie S, Tian B, Wang X, Pincus DH, Welker M, Gilhuley K, et al. Fusobacterium nucleatum subspecies identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2015;53:1399-402.
29. Kim D, Ji S, Kim JR, Kim M, Byun JH, Yum JH, et al. Performance evaluation of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, ASTA MicroIDSys system, in bacterial identification against clinical isolates of anaerobic bacteria. Anaerobe 2020;61:102131.
30. Fedorko DP, Drake SK, Stock F, Murray PR. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis 2012;31:2257-62.
31. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543-51.
32. Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, et al. Benchmarking of methods for genomic taxonomy. J Clin Microbiol 2014;52:1529-39.
33. Park SN, Lim YK, Shin JH, Kim HS, Jo E, Lee WP, et al. Fusobacterium pseudoperiodonticum sp. nov., isolated from the human oral cavity. Curr Microbiol 2019;76:659-65.
34. Branda JA, Fritsche TR, Burnham CA, Butler-Wu S, Doern C, Doing KM, et al. CLSI guideline M58. Methods for the identification of cultured microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. 1st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
35. Kook JK, Park SN, Lim YK, Cho E, Jo E, Roh H, et al. Genome-based reclassification of Fusobacterium nucleatum subspecies at the species level. Curr Microbiol 2017;74:1137-47.
36. Claydon MA, Davey SN, Edwards-Jones V, Gordon DB. The rapid identification of intact microorganisms using mass spectrometry. Nat Biotechnol 1996;14:1584-6.
37. Martiny D, Busson L, Wybo I, El Haj RA, Dediste A, Vandenberg O. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:1313-25.
38. Lee Y, Sung JY, Kim H, Yong D, Lee K. Comparison of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform, ASTA MicroIDSys, with Bruker Biotyper for species identification. Ann Lab Med 2017;37:531-5.
39. Jung J, Kim SY, Park YJ, Lee J, Suk HS, Ha SI, et al. Comparison of the ASTA MicroIDSys and VITEK MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems for identification of clinical bacteria and yeasts. J Infect Chemother 2020;26:1328-33.