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Original Article

Identification of *Demodex Hominis* using Matrix-Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry

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Abstract:
This study investigated the application of Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) to identify Demodex hominis at various developmental stages and establish a reproducible spectral database for Demodex hominis. The profile spectra of *Demodex hominis* were obtained by VITEK® Mass Spectrometry (MS) and the database was created by the SARAMIS™ super spectra tool. In the validation study and blind test, correct identification at the species and development stage level is based on SARAMIS™ software analysis. Consistent and reproducible MS profiles were obtained in 28 specimens. A spectrum database, including 4 super spectra, revealed 15 discriminating mass peaks. Peaks 4304.7 and 8609.8 were specific peaks for *Demodex hominis*. Peaks 3346.8 and 5236.6 are shown in adult *Demodex brevis* spectra. Similarly, peaks 3788.2 and 7673.3 emerged in the larva *Demodex brevis*. Peaks 4109.6, 5383.2, and 7518.3 presented in adult *Demodex follicurolum* spectra. Peak 4696.8 appeared only in larva *Demodex follicurolum*. In the blind test, 44 of the 48 demodex specimens in the target development stage were correctly identified. These findings highlight that protein profiling utilizing MALDI-TOF-MS represents an efficient and reliable methodology for the identification of Demodex hominis across both species and developmental stages. An initial database comprising the spectral data of the two Demodex hominis species in China has been established.

Keywords: *Demodex hominis*, mites, eyelash, mass spectrometry, matrix-assisted laser desorption/ionization.

Introduction

Demodex is the genus of a small parasitic mite residing in or near the hair follicles and sebaceous glands of the skin of mammals, belonging to the kingdom Animalia, phylum Arthropoda, class Aracnida, subclass Acari, and genus: Demodex. To date, up to 140 species and subspecies have been identified. Species names typically relate to the animals they parasitize. The two species in humans, Demodex folliculorum and Demodex brevis, might more appropriately be classified as Demodex hominis, which is frequently referred to as eyelash mites. Demodex mites serve as numerous disease vectors in both animals and humans, such as dermatitis, rosacea, chronic blepharitis, and meibomian gland dysfunction. [1-4].

In the clinical aspect, the classification of *Demodex hominis* has been mainly based on their morphological characteristics, especially body-to-tail ratio[5] (**Figure 1**).

Figure 1. Microscopic features of different development stage demodex hominis. These *Demodex* are divided into head–neck and body–tail parts, with 8 short legs attached to the former body segment. The body-to-tail ratio is 1:2 to 1:4 for *Demodex folliculorum* but close to 1:1 for *Demodex brevis*. A. Adult and larva *Demodex folliculorum.* B. Adult *Demodex brevis*. C. Larva and ovum *Demodex folliculorum.* D. Larva *Demodex brevis.*

Nevertheless, the morphology of *Demodex* hominis can be readily influenced by environmental factors, presenting considerable plasticity and phenotypic variances, even during diverse developmental stages. Furthermore, the coexistence of two or more *Demodex* species in one host poses challenges and uncertainties to morphological classification. The encouraging aspect is that the evolution of molecular biology technology and the nascent DNA barcoding technique in recent decades can be employed for the molecular classification, identification, and phylogenetic study of *Demodex* [6]. Molecular methods, whichever is available, are accurate and applicable to any development stages of *Demodex*, but they are time-consuming, labor-intensive, and high-cost.

In recent years, Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) has been examined as an effective and accurate way for identifying microorganisms in both research and clinical fields. This approach has been developed to achieve high-throughput, accurate and reproducible identification of clinically relevant microorganisms like bacteria, yeasts and filamentous fungi at a low cost and with minimal preparation time. [7, 8] Additionally, this proteomic approach has been employed for the identification of parasites (fleas, ticks)[9, 10] and insects (drosophila[11] and mosquitoes[12]). Therefore, the MALDI-TOF-MS approach is a promising technique for clarifying the relationships within species and between closely related species. However, studies about identifying *Demodex Hominis* by MALDI-TOF-MS are limited. Thus, the aim of our study was to explore the application of MALDI-TOF-MS for the identification of *Demodex hominis* at different developmental stages and to discover a reliable approach to obtain reproducible spectra, with the goal of establishing a *Demodex hominis* database.

Materials and methods

Demodex collection

The sampling of eyelashes for *Demodex* examination was conducted as we previously reported [13, 14]. Three eyelashes, isolated and taken from the upper lids of a healthy subject by sterile forceps, were placed on a glass slide. A drop of sterile pure water was mounted on the end of the eyelash looking for *Demodex*. Specimens were collected in three stages of *Demodex* (Figure 1), including 140 *Demodex folliculorum* (15 ova, 50 larvae, and 75 adults) and 100 *Demodex brevis* (5 ova, 30 larvae, and 65 adults). *Demodex* specimens were preserved in pure water for a short time. *Demodex* species identification was

used in morphological identification[15] under the supervision of a medical entomologist.

Sample preparation for MALDI-TOF-MS

For the MALDI-TOF-MS analysis preparation, the fresh specimens were rinsed with distilled water. Five *Demodex* (ovum, larva, or adult) as a sample were triturated by tissue grinder (OSE-Y20 TIANGEN BIOTECH Beijing China) for 5 minutes. After centrifugation (12000 rpm 30 minutes), 1µl of supernatant and 1µl of CHCA matrix solution (bioMérieux, France) were loaded on a MALDI-TOF-MS FlexiMass-DS disposable target slide (Cat. #: 149 TO-430) to three spots for each specimen and air dried. E. coli ATCC 8739 served as a calibrated control in the acquisition.

MALDI-TOF-MS analysis

The protein mass profiles were acquired using MALDI-TOF Vitek Mass Spectrometry (VITEK® MS RUO, bioMérieux, Marcy l'Etoile, France) in the mass range of 2–20 kDa. Spectra were acquired on a Vitek MS instrument with a nitrogen laser (337 nm) operating in positive linear mode with delayed extraction at a 58 kV accelerating voltage. Each spectrum was automatically collected in the positive ion mode as an average of 500 laser shots (five laser shots at 100 profiles per sample). The resulting profiles were analyzed using Saramis premium software (SARAMIS™ version 4.0.0.14). Peak lists were trimmed to a mass range of 2–20 kDa, and peaks with a relative intensity below 5% were removed. Peak lists were binned, and average masses were calculated using the SARAMIS™ super spectrum tool. Spectra, whose dendrogram similarities were more than 60%, containing 100 to 300 peaks were retained for analysis. Only the reproducible spectra (consensus spectra> 80%) were loaded into the SARAMIS™ super spectra tool to create a super spectral database with 14 specimens (6 larvae and 8 adults) for *Demodex folliculorum* and 14 specimens (6 larvae and 8 adults) for *Demodex brevis*.

Species identification

The identification was validated by a blind test using new fresh *Demodex* samples of the two species. Three to five larva or adult *Demodex* specimens of each species were examined against the database established in the SARAMIS™ software. Each specimen was coded before beginning the blind test. The threshold for identification was set at confidence 75% of biomarker matches based on the reference data set under SARAMIS™ Premium user guidelines.

Results

MALDI-TOF-MS spectrum analysis and database assembly

A total of 48 flea specimens underwent the MALDI-TOF/MS premium analysis. The analysis of the spectral profiles using the SARIMAS software revealed that the spectra obtained from homogenized supernatant offered consistent and reproducible spectral profiles with peaks of high intensities in the range of 3–12 kDa (**Figure 2**), with 198-411 peaks per spectrum.

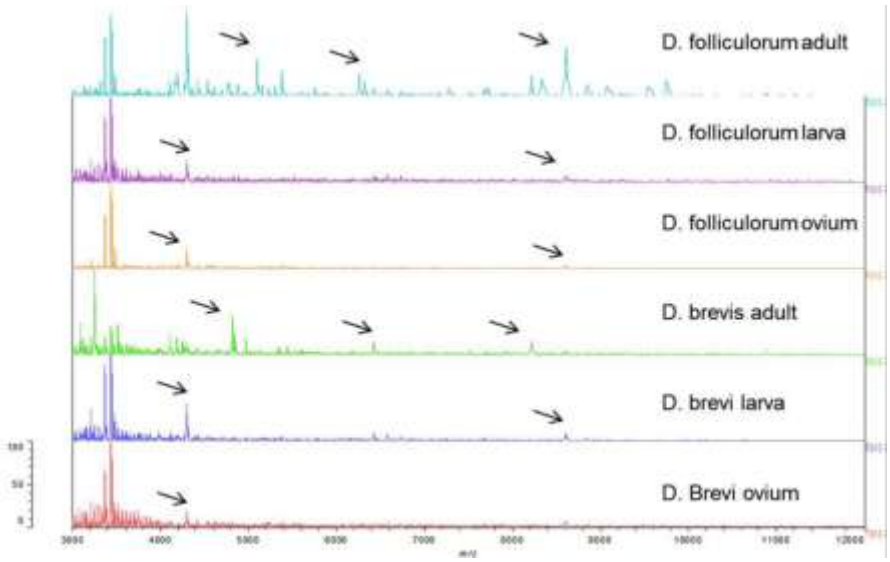

Figure 2. Vitek Mass Spectrometry (VITEK® MS) spectra in the range of 3–12 k Da of different stages for *Demodex hominis*. Four super spectra were created for the larva and adult stages. Most

specific peaks are indicated by arrows.
(Abbreviations: *D. folliculorum=Demodex folliculorum; D. brevis =Demodex brevis*)

The spectra for each species of ticks were demonstrated to be reproducible for all tested groups after spectral analysis. This reproducibility was witnessed for both the *Demodex folliculorum* and *Demodex brevis* specimens. Subsequently, a database was established and loaded with two species of 28 larva and adult specimens. The protein profiles of 14 specimens for each species were utilized to compile the total mass spectra. The resulting dendrogram of species was clustered with distinct branches (**Figure 3**).

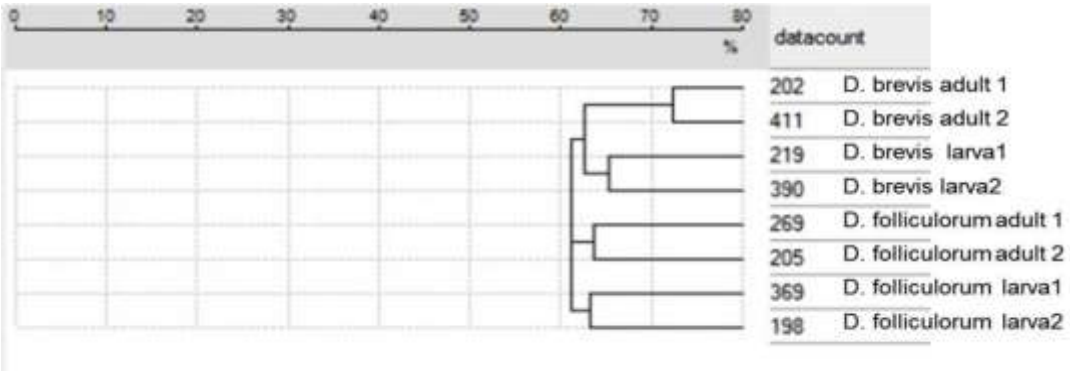

Figure 3. Dendrogram of Vitek Mass Spectrometry (VITEK® MS) spectra in the range of 3–12 k Da from two individual species of *Demodex hominis*.
(Abbreviations: *D. folliculorum=Demodex folliculorum; D. brevis =Demodex brevis*)

Additionally, four super spectra were generated for *Demodex folliculorum* and *Demodex brevis* in adult and larva stages.

Study validation using a blind test.

The accuracy of the Demodex hominis reference database was determined in the second step via a validation study. A comparison of all these specimens within the database using VITEK® MS software produced satisfactory outcomes. The correct identification was accomplished at the species level, with a confidence index ranging from 90% to 99.9% similarity. In the blind test, 15 adult or larva specimens were compared against the database for species identification. All specimens were precisely identified, and the confidence was higher than 90%, corresponding to significant identification (**Table 1** and **Table 2**). Distinct protein profiles were obtained in accordance with their developmental stage (**Figure 2**). Therefore, the spectra from the supernatant were employed to establish the database and subsequently evaluated in a blind test.

Table 1. Validation of VITEK® MS of 15 *Demodex hominis* in different stages.

Species stage	Number	Correspondence of MALDI-TOF to morphological identification (%)	Confidence rang
Demodex brevis adult	15	13/15 (86.67)	75-99.9
Demodex brevis larva	10	10/10 (100.00)	90-99.9
Demodex folliculorum adult	13	12/13 (92.31)	70-99.9
Demodex folliculorum larva	10	9/10 (90.00)	70-99.9

Abbreviations: VITEK® MS, Vitek Mass Spectrometry. MALDI-TOF, Matrix-assisted laser desorption/ionization time of flight.

Table 2. Comparison of MALDI-TOF *Demodex hominis* in different stages, ranging from 3-20 k Da.

Mass m/z (Da)	***Demodex brevis* adult**	***Demodex brevis* larva**	***Demodex folliculorum* adult**	***Demodex folliculorum* larva**
3187.7	yes	yes	no	no

3278.6	no	no	yes	yes
3346.8	yes	no	no	no
3788.2	no	yes	no	no
4109.6	no	no	yes	no
4304.7	yes	yes	yes	yes
4696.8	no	no	no	yes
5236.6	yes	no	no	no
5383.2	no	no	yes	no
6426.2	no	no	yes	yes
6581.8	yes	yes	no	no
7518.3	no	no	yes	no
7673.3	no	yes	no	no
8609.8	yes	yes	yes	yes
8865.1	no	no	yes	yes

Abbreviations: MALDI-TOF, Matrix-assisted laser desorption/ionization time of flight.

Discussion

The Demodex are recognized as vectors of several human diseases and are thereby regarded as having public health significance worldwide[1, 16]. The present study affirmed that specimen protein extracts are adequate for the identification of arthropods by using the MALDI-TOF-MS approach when a corresponding species spectrum has already been uploaded in the reference database. Our results indicated that the utilization of the homogenate of an entire demodex body was a reliable sample for species discrimination through the MALDI-TOF-MS approach. These findings are in accordance with those of previous identifications of arthropod species based on Drosophila [11], ticks[17, 18], Culicoides[19], and mosquito species[20, 21] using proteins extracted from either the entire body or body sections of individual specimens. In our case, the spectral profiles generated from the fresh entire body and subjected separately to MALDI-TOF-MS were reproducible. These results are consistent with previous studies reporting that fresh samples disclosed a higher average data count than specimens stored for a long time in 70% ethanol.[22]

Our study utilized MALDI-TOF-MS for the identification of *Demodex hominis* and established the first database reference of *Demodex hominis* in China. The established database allows for cost-effective and rapid identification of *Demodex* species and different stages with high specificity, in contrast to molecular identification[6]. We verified that the application of the VITEK® MS system in this study generated high-quality spectra with the CHCA matrix. Generally, MS offers excellent identification of *Demodex*, yielding satisfactory results for the species under investigation.

Conclusion

In summary, the present study discloses that MALDI-TOF-MS is an efficient and dependable approach for the prompt identification of *Demodex*. Further investigations are requisite to explore the spectra of other mite species. Nevertheless, a preliminary database encompassing the spectra of the two species of *Demodex hominis* has been established, and the database will be updated on a regular basis with new fresh mite specimens. When comparing morphological and molecular methods, MALDI-TOF-MS is relatively straightforward and economical subsequent to obtaining the instrument, with no requirement for costly chemicals. MALDI-TOF-MS holds potential as a reference method for *Demodex* identification and could evolve into an economically beneficial diagnostic tool for mites.

Data availability: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

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Conflicts of Interest: The authors declare no conflicts of interest relevant to this article.

References

1. Morrás, P.G., et al., Rosacea-like demodicidosis in an immunocompromised child. Pediatric Dermatology, 2003. 20(1): p. 28–30.
2. Holmes, A.D., Potential role of microorganisms in the pathogenesis of rosacea. Journal of the American Academy of Dermatology, 2013. 69(6): p. 1025.
3. Liang, L.Y., et al., Ocular demodicosis as a potential cause of pediatric blepharoconju nctivitis. Cornea, 2010. 29(12): p. 1386.
4. Luo, X., et al., Ocular Demodicosis as a Potential Cause of Ocular Surface Inflammation. Cornea, 2017: p. 1.
5. Cheng, A.M., H. Sheha, and S.C. Tseng, Recent advances on ocular Demodex infestation. Current Opinion in Ophthalmo logy, 2015. 26(4): p. 295.
6. Zhao, Y.E., et al., Molecular identification and phylogenetic study of Demodex caprae. Parasitology Research, 2014. 113(10): p. 3601-8.
7. Mellmann, A. and F.C. Bimet, High interlaboratory reproducibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry-based species identification of nonfermenting bacteria. Journal of Clinical Microbiology, 2009. 47 (11): p. 3732.
8. Santos, C., et al., Filamentous fungal characterizations by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Journal of Applied Microbiology, 2010. 108(2): p. 375-85.
9. Murugaiyan, J. and U. Roesler, MALDI-TOF MS Profiling-Advances in Species Identification of Pests, Parasites, and Vectors. Frontiers in Cellular & Infection Microbiology , 2017. 7.
10. Singhal, N., M. Kumar, and J.S. Virdi, MALDI-TOF MS in clinical parasitology: applications, constraints and prospects. Parasitology, 2016. 143(12): p. 1491.
11. Feltens, R., et al., Discrimination of different species from the genus Drosophila by intact protein profiling using matrix-assisted laser desorption ionization mass spectrometry. Bmc Evolutionary Biology, 2010. 10(1): p. 1-15.
12. Laroche, M., et al., MALDI-TOF MS as an innovative tool for detection of Plasmodium parasites in Anopheles mosquitoes. Malaria Journal, 2017. 16(1): p. 5.
13. Bhandari, V. and J.K. Reddy, Blepharitis: always remember demodex. Middle East African Journal of Ophthalmology, 2014. 21 (4): p. 317.
14. Gao, Y.Y., et al., High prevalence of Demodex in eyelashes with cylindrical dandruff. Investigative Ophthalmology & Visual Science, 2005. 46(9): p. 3089.
15. Desch, C. and W.B. Nutting, Demodex folliculorum (Simon) and D. brevis Akbulatova of man : redescription and reevaluation. Journal of Parasitology, 1972. 58(1): p. 169.
16. Erbagci, Z., I. Erbagci, and S. Erkiliç, High incidence of demodicidosis in eyelid basal cell carcinomas. International Journal of Dermatology, 2003. 42(7): p. 567–571.
17. Yssouf, A., et al., Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Identification of Tick Vectors. Journal of Clinical Microbiology, 20 13. 51(2): p. 522-8.
18. Karger, A., et al., Species determination and characterization of developmental stages of ticks by whole-animal matrix-assisted laser desorption/ionization mass spectrometry. Ticks and Tick-borne Diseases, 2012. 3(2): p. 78-89.
19. Sambou, M., et al., Comparison of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and Molecular Biology Techniques for Identification of Culicoides (Diptera: Ceratopogonidae) Biting Midges in Senegal. Journal of Clinical Microbiology, 2015. 53(2): p. 410-8.
20. Yssouf, A., et al., Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry: An Emerging Tool for the Rapid Identification of Mosquito Vectors. Plos One, 2013. 8(8): p. e72380.
21. Müller, P., et al., Identification of Cryptic Anopheles Mosquito Species by Molecular Protein Profiling. Plos One, 2013. 8(2): p. e57 486.
22. Yssouf, A., et al., Identification of flea species using MALDI-TOF/MS. Comparative Immunology Microbiology & Infectious Diseases, 2014. 37(3): p. 153-157.