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# Comparison of the Vitek MS and Bruker Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of *Rhodococcus equi* and *Dietzia* spp.

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**ABSTRACT** *Rhodococcus equi* causes pyogranulomatous pneumonia in domesticated animals and immunocompromised humans. *Dietzia* spp. are environmental bacteria that have rarely been associated with human infections. *R. equi* and *Dietzia* spp. are closely related actinomycetes. Phenotypic discrimination between *R. equi* and *Dietzia* on the basis of their Gram stain morphology and colony appearance is problematic. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a fast, reliable, and cost-effective method for identification of a wide variety of microorganisms. We have evaluated the performance of Bruker Biotyper versus that of Vitek MS for identification of a collection of 154 isolates identified at the source as *R. equi* that includes isolates belonging to the genus *Dietzia*. PCR amplification of the *choE* gene, encoding a cholesterol oxidase, and 16S rRNA sequencing were considered the reference methods for *R. equi* identification. Biotyper identified 131 (85.1%) of the 154 isolates at the species level, and this figure increased to 152 (98.7%) when the species cutoff was reduced from a score of ≥2.000 to ≥1.750. Vitek MS correctly identified at the species level 130 (84.4%) isolates as long as bacteria were extracted with ethanol but only 35 (22.7%) isolates when samples were prepared by direct extraction from colonies. The two systems allowed differentiation between *R. equi* and *Dietzia* spp., but identification of all *Dietzia* sp. isolates at the species level needed sequencing of the 16S rRNA gene.

**KEYWORDS** *Rhodococcus equi*, *Dietzia* spp., identification, MALDI-TOF MS, genotypic identification

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The soil-dwelling, saprophytic actinomycete *Rhodococcus equi* is a multihost, facultative intracellular pathogen that causes pyogranulomatous infections in several species of domesticated animals, in particular, in young foals and pigs (1). In addition, *R. equi* infects humans (2). At-risk groups are immunocompromised people, such as HIV-AIDS or cancer patients, and transplant recipients. Infection with *R. equi* in these patients results in a granulomatous form of pneumonia whose symptoms resemble clinical and pathological signs of pulmonary tuberculosis and which has a high mortality rate. *Dietzia* spp. are environmental bacteria that have been associated with human infections in a small number of cases (3). The Gram morphology and colony appearance of the species of the genus *Dietzia* are remarkably similar to those seen with *R. equi*.

In clinical microbiology laboratories, *R. equi* is routinely identified using a conventional approach based on growth characteristics, morphology of colonies, the CAMP

**TABLE 1** Comparison of the identification results for the 154 isolates with the Vitek MS and the Biotyper MALDI-TOF MS systems using two different methods for the preparation of the samples[a]

| MALDI-TOF MS system | No. of isolates identified by: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Direct colony method | | | | Extraction method | | | |
| | Total | *R. equi* | *Dietzia* spp. | Not identified | Total | *R. equi* | *Dietzia* spp. | Not identified |
| Vitek MS | 35 | 35 | 0 | 119 | 130 (35 + 95*) | 124 (35 + 89*) | 6 (0 + 6*) | 24 |
| Bruker Biotyper | 141 | 141 | 0 | 13 | 153 (141 + 12*) | 144 (141 + 3*) | 9 (0 + 9*) | 1 |

[a]*, the number of strains which required sample extraction to give a reliable identification.

reaction, and biochemical tests, which are frequently performed using the API Coryne system (bio-Mérieux, Marcy l'Etoile, France), a commercial multisubstrate kit that includes *R. equi* in its database (4). However, this method is time-consuming and its reliability for the identification of rhodococcal isolates is low, resulting in misidentification of *R. equi* as another rhodococcal species or even another actinomycetes (5). For instance, Bemer-Melchior et al. (6) reported that API Coryne V2.0 gave an identification of *R. equi* for a *Dietzia maris* isolate obtained from an immunocompromised patient. Our group (7) and other authors (8) have also reported the misidentification of *Dietzia* strains as *R. equi*. PCR methods targeting the *R. equi choE* gene, encoding a cholesterol oxidase, are accurate and reliable, allowing identification of *R. equi* isolated from human, animal, or environmental samples (9, 10).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a fast, highly accurate, and cost-effective method for identification of bacterial and fungal pathogens (11). The MALDI Biotyper (Bruker Daltonics GmbH, Bremen, Germany) and the Vitek MS (bioMérieux) platforms are the two MS-based platforms more commonly used for microbial identification. In a previous study (12), we reported that the Bruker MS system identified successfully at the species level a group of 11 isolates initially assigned to *R. equi* by API Coryne V2.0, confirming identification of all isolates as *R. equi* except 1, which was classified as *Dietzia maris*. Although limited to a few isolates, that study envisaged that MALDI-TOF MS might represent an alternative, fast, and worthwhile method for a reliable identification of *R. equi* and *Dietzia* spp.

In the present study, we carried out a comparative evaluation of the performance of Vitek MS versus that of Bruker Biotyper for identification of a panel of 154 isolates initially assigned to *R. equi* by API Coryne V2.0 that included isolates belonging to the genus *Dietzia*.

## RESULTS

A total of 144 of the 154 isolates were positive in the PCR amplification assay targeting the *choE* and therefore were assigned to *R. equi*. The remaining 10 isolates gave a negative *choE* PCR result. By partial 16S rRNA sequencing, they were identified as *Dietzia maris* ($n = 5$), *Dietzia natronolimmnaea* ($n = 3$), and *Dietzia cinnamea* ($n = 2$). This identification was considered the reference method.

In direct testing of bacterial colonies with Vitek MS, the system correctly identified 35 (22.7%) isolates at the species level at its best performance. In most cases, failure of the system to give a precise identification was due to its inability to read the samples. When bacteria were extracted with ethanol, Vitek MS correctly identified at the species level a total of 130 (84.4%) isolates: 124 were identified as *R. equi* and 6 as *Dietzia* spp. (Table 1). Within the collection of 144 *R. equi* isolates, Vitek MS properly identified 124 (86.1%) isolates with the maximum score (99.9%). When the confidence score was decreased to 50%, an additional isolate was correctly identified as *R. equi* as well. Considering the group of 10 *Dietzia* sp. isolates, Vitek MS identified 6 (60%) strains with the maximum score (99.9%) and 2 (20%) strains (*D. natronolimnaea* and *D. maris*) with a score of 50%, whereas the remaining 2 strains (*D. natronolimnaea* and *D. cinnamea*) were not identified (Table 2).

The Biotyper provided high-confidence identifications without extraction of the

**TABLE 2** Identification results for *R. equi* ($n = 144$) and *Dietzia* spp. ($n = 10$) by Vitek MS versus Bruker Biotyper

| Microorganism(s) | No. of strains | No. (%) of isolates with indicated result by: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Vitek MS | | | | Bruker Biotyper | | | |
| | | Correct identification at species level | Correct low score (50%–99.8%) | Not identified | Identification at genus level | Correct identification at species level | Correct low score (1.7–2) | Not identified | Identification at genus level |
| *R. equi* | 144 | 124 (86.1) | 1 (0.7) | 19 (13.2) | 0 | 130 (90.3) | 14 (9.7) | 0 | 0 |
| *Dietzia* spp. | 10 | 6 (60) | 2 (20) | 2 (20) | 0 | 1 (10) | 7 (70) | 1 (10) | 1 (10) |
| *D. maris* | 5 | 4 | 1 | 0 | 0 | 1 | 4 | 0 | 0 |
| *D. natronolimnaea* | 3 | 1 | 1 | 1 | 0 | 0 | 3 | 0 | 0 |
| *D. cinnamea* | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| Total no. (%) of strains | 154 | 130 (84.4) | 3 (1.9) | 21 (13.6) | 0 | 131 (85.1) | 21 (13.6) | 1 (0.6) | 1 (0.6) |

samples for 141 (91.6%) isolates. Conversely, a group of 13 isolates, including the 10 *Dietzia* sp. isolates and 3 *R. equi* isolates, required acetonitrile extraction (Table 1). The system correctly identified at the species level 131 (85%) isolates (130 *R. equi* and 1 *Dietzia* sp.), giving scores of ≥2.000 in all cases. The 130 isolates identified as *R. equi* were positive for PCR *choE*, whereas the isolate identified as a *Dietzia* sp. was negative. The remaining *R. equi* isolates ($n = 14$), giving scores in the range of 1.700 to 1.900, were reanalyzed using a species-level cutoff value of ≥1.750. With the ≥1.750 score cutoff, all 14 of these isolates were also identified as *R. equi*, in accordance with the results of PCR amplification of the *choE* gene (all were positive). Considering the group of 10 *Dietzia* sp. isolates, the Biotyper identified 1 (10%) strain (score, ≥2.000) at the species level and 8 (80%) strains at the genus level (scores ranged between 1.700 and 1.999) and another 1 (10%) could not be identified (score, <1.700). When the species level cutoff value was lowered to a score of ≥1.750, 7 of 8 *Dietzia* spp. were also correctly identified at the species level, as subsequently confirmed by 16S rRNA sequencing (Table 2).

## DISCUSSION

Identification of *R. equi* by conventional methods based on colony morphology, growth properties, or biochemical profiles is not reliable and requires molecular methods to differentiate it from *Dietzia* spp. MALDI-TOF MS is a fast method for identification of microorganisms of clinical interest and could be an adequate option in considering *R. equi* and *Dietzia* spp. In routine diagnostics, MALDI-TOF MS has advantages with respect to the time and effort needed to obtain identification results at the species level. However, there is little information on the performance of the two main commercially available MALDI-TOF MS systems for the identification of these two closely related actinomycetes.

Overall, with the direct colony extraction method, the Biotyper permitted the identification of 91.6% of the strains tested, in contrast to only 22.7% for Vitek MS. For the Biotyper system, previous studies have reported improved species-level identification rates without an increase in the false-identification rates for a variety of different organisms by decreasing the identification score either to 1.900 (13) or to 1.700 (14). By lowering the score representing species-level identification from ≥2.000 to ≥1.750, we were able to identify at the species level practically all the strains tested in this study, 144 (100%) *R. equi* strains and 8 (80%) of 10 *Dietzia* sp. strains, without any misidentification. Only one strain gave a score lower than 1.700 and therefore could not be identified with the Biotyper MS. This strain was identified as *D. cinnamea* by 16S rRNA sequencing. *D. cinnamea* was first isolated from a perianal swab of a patient with a bone marrow transplant (15), but it is mostly known for its ability to degrade a wide variety of organic compounds and rarely for its pathogenic potential (16). The performance of the Biotyper in identification of *R. equi*, distinguishing it from *Dietzia* spp., is comparable to the performances of PCR *choE* and 16S rRNA sequencing. When the species cutoff value was reduced to a score of ≥1.750, Biotyper correctly identified all the *R. equi* isolates. PCR *choE* analysis is a reliable method for *R. equi* identification, but MALDI-TOF MS is faster and more economical in terms of both labor and monetary cost. Although *Dietzia* spp. are not commonly associated with human infections, sporadic cases necessitate accurate identification of *Dietzia* spp. at the species level. 16S rRNA sequencing is currently the only method that guarantees definitive and conclusive identification of *Dietzia* spp. to the rank of species.

The results obtained in this study indicate that for the Vitek MS system, pretreatment of bacterial colonies with ethanol improves substantially the number of correct identifications with the highest level of confidence. Actinomycetes such as *R. equi* and *Dietzia* spp. contain in their cell walls long-chain mycolic acids as well as a great amount of lipopolysaccharide, preventing the complete uptake of energy when fired upon by the laser. Consequently, mass spectra are not generated. With the use of ethanol in the extraction method, a substantial amount of mycolic acids and polysaccharide is eliminated, while the target proteins are preserved, allowing the generation of high-quality

spectra (17). We consider that, with the extraction method, the Vitek MS system has performed properly, with an acceptable rate of 84.4% of samples correctly identified at the species level. However, it was not able to identify 20 *R. equi* isolates or to distinguish properly 4 *Dietzia* spp. from *R. equi*, while PCR *choE* analysis correctly identified all the *R. equi* isolates. The SARAMIS 2.0 database includes three species of the genus *Dietzia*: *D. maris*, *D. natronolimnaea*, and *D. cinnamea*. However, Vitek MS identified at the species level only 6 of the 10 *Dietzia* isolates analyzed. The genus *Dietzia* has been established recently, and only a few strains have been implicated in human infections (18).

Overall, in the comparisons of the two systems, the MALDI Biotyper system provided higher accuracy than the Vitek MS system for identification of *R. equi* as well as for differentiation of *R. equi* from *Dietzia* spp. Although the data were limited in that only 10 isolates were included in the study, the results indicated that proper identification of *Dietzia* spp. at the species level requires confirmation by 16S rRNA sequencing. Spectral databases should be regularly updated by suppliers to improve identification rates.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 154 isolates, all originally identified as *R. equi*, were included in the study. A total of 129 of the 154 isolates were previously identified as *R. equi* by means of PCR amplification of the *choE* gene, encoding a *R. equi* cholesterol oxidase (9). Eight of the 154 isolates were negative for the PCR *choE* gene and were identified as members of the genus *Dietzia* by 16S rRNA sequencing (7). The remaining 17 isolates (15 *R. equi* isolates and 2 *Dietzia* sp. isolates) were submitted to the clinical microbiology laboratory of Marqués de Valdecilla university hospital during the period 2010 to 2014. The collection included strains isolated from AIDS patients at different Spanish hospitals ($n = 71$), isolates from pneumonic foals ($n = 34$), and strains isolated from soil samples collected from the environment ($n = 49$). They were grown on Columbia horse blood agar plates at 37°C and stored in cryogenic vials at −80°C in brain heart infusion broth with 20% glycerol.

**Reference method.** We consider the *choE*-based PCR to be the reference method for *R. equi* identification. All the strains were assayed by *choE* PCR, as described in detail elsewhere (9). PCR-negative *choE* isolates were identified by sequencing of a species-specific region of the 16S rRNA gene delimited by primers DG74 (AGGAGGTGATCCAACCGCA) and PLO6 (5′-GGTTAAGTCCCGCAACGAGCGC-3′) (19). The PCR products were sequenced using an ABI Prism 377 apparatus (Applied Biosystems, Foster City, CA, USA). Homology searches were performed with the BLAST tool at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

**Vitek MS.** Two different extraction protocols were used for the preparation of the samples: (i) direct extraction from colonies and (ii) extraction with ethanol. For the direct colony extraction procedure, a portion of a fresh colony was directly applied as a thin film on a spot of a MALDI disposable target plate, dried, and overlaid with 1 $\mu$l of matrix and 0.6 $\mu$l of 28.9% formic acid solution (Vitek MS-FA). Upon drying, the target plate was loaded into the Vitek MS instrument for identification. For the ethanol extraction procedure, a single colony was transferred to a 1.5-ml Eppendorf tube containing 300 $\mu$l of API suspension medium (bioMérieux) and mixed thoroughly using a vortex mixer. Absolute ethanol (Merck, Darmstadt, Germany) (900 $\mu$l) was added, and the mixture was mixed thoroughly again. After centrifugation at 13,000 rpm was performed for 2 min, the supernatant was discarded and the pellet resuspended in the residual ethanol. A volume of 1 $\mu$l of the resuspended pellet was loaded on the steel target plate, dried, and overlaid with 1 $\mu$l of Vitek MS-CHCA matrix (a saturated solution of $\alpha$-cyano-4-hydroxycinnamic acid [HCCA]–acetonitrile/ethanol/water [1:1:1] acidified with 3% [vol/vol] trifluoroacetic acid) and again allowed to air dry.

Each strain tested was spotted twice on the same target slide. Measurements were performed on a Vitek MS mass spectrometer (bioMérieux) according to the manufacturer's suggested settings using automatically collected spectra. Captured spectra were analyzed with Shimadzu Launchpad identification software that included the database SARAMIS MS-ID version 2.0 (Anagnos Tee GmbH). For each run, *Escherichia coli* ATCC 8739 was included as a standard to calibrate the instrument and to validate the run. The system reported the best identification matches along with confidence values from 0% to 100%. For a reliable identification at the species level, only hits with scores of 99.9% were accepted. Peak matches that yielded identification results with confidence values between 50% and 99.8% were considered acceptable but required confirmation by 16S rRNA sequencing. When a good identification was not obtained with the direct colony extraction method, subsequent analysis was done with the ethanol extraction method.

**Bruker Biotyper.** All the strains were subjected to analysis with the system using the following procedure: the bacterial biomass was smeared onto a metallic MALDI-TOF MSP 96 plate (Bruker Daltonics) and covered with 1 $\mu$l of saturated $\alpha$-cian-4-hydroxycinamic acid–50% acetonitrile–2.5% trifluoroacetic acid. The plate was dried at room temperature for 5 min. When the first attempt at identification did not give results with a high level of confidence, samples were identified using formic acid-acetonitrile extraction (20). Briefly, 2 to 3 colonies were transferred to a 1.5-ml Eppendorf tube containing 300 $\mu$l of distilled water and were then mixed with 900 $\mu$l of ethanol by pipetting. The

suspension was pelleted by centrifugation at 13,000 rpm for 2 min, evaporated to dryness, and then reconstituted in 50 μl of 70% formic acid (Sigma-Aldrich, Madrid, Spain). After incubation for 30 s, 50 μl of acetonitrile (Sigma-Aldrich) was added. The suspension was then centrifuged at 13,000 rpm for 2 min. Next, 1.0 μl of the supernatant was applied to a 96-spot polished steel target plate (Bruker Daltonics) and dried. A saturated solution of 1.0 μl of MALDI matrix (α-cyano-4-hydroxycinnamic acid [HCCA]; Bruker Daltonics) was applied to each sample and dried. Measurements were performed with a Bruker Microflex LT MALDI-TOF MS system (Bruker Daltonics) using FlexControl software with Compass Flex Series version 1.3 software and a 60-Hz nitrogen laser (337-nm wavelength). The protein spectra with mass-to-charge ratios (*m/z*) ranging from 2,000 to 20,000 were explored with the Bruker Biotyper 3.1 software package with default settings and compared with the reference spectra present in the database, showing the 10 most similar patterns for each isolate. The database for identification was the reference Biotyper library v4.0 5,627 MSP (Bruker Daltonic), which included 5,627 species. The similarity of patterns was represented as a score (≥2.000, identification at the species level; 1.700 to 1.999, identification at the genus level; <1.700, no reliable identification). *E. coli* ATCC 25922 was used as a standard for calibration and as a reference for quality control.

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