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# Development of a database for the rapid and accurate routine identification of *Achromobacter* species by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS)

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1 **Original article**

2 **Title**

3 Development of a database for the rapid and accurate routine identification of *Achromobacter* species by  
4 Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

5

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24

25 °We need the collaborators to be tagged for PubMed purposes. In print we would like a link to a footnote  
26 on the first page.

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28 Length of the abstract: 252 words

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## 33 **Abstract**

### 34 **Objectives:**

35 *Achromobacter* spp. are emerging bacteria in respiratory samples from cystic fibrosis patients. The  
36 current reference methods (*nrdA*-sequencing or MultiLocus Sequence Typing) can identify 18 species  
37 which are often misidentified as *A. xylosoxidans* by conventional techniques. Few studies have suggested  
38 that MALDI-TOF/MS provides accurate identification of genus but not of species.

39 The aims of this study were 1) to generate a database for MALDI-TOF/MS Bruker including the 18  
40 species, 2) to evaluate the suitability of the database for routine laboratory identification, and 3) to  
41 compare its performance with that of the currently available Bruker default database.

### 42 **Methods:**

43 A total of 205 isolates belonging to the 18 species identified by *nrdA* sequencing were used to build a  
44 local database. Main spectra profiles (MSPs) were created according to Bruker's recommendations for  
45 each isolate with the Biotyper software.

46 Performance of the default Bruker database and ours for routine use were compared by testing 167 strains  
47 (including 38 isolates used from MSP creation) belonging to the 18 species identified by *nrdA*-sequencing  
48 from direct colonies cultivated on various media.

### 49 **Results:**

50 Our new database accurately identified 99.4% (166/167) of the isolates from the 18 species (score  $\geq 2.0$ )  
51 *versus* only 50.9% (85/167) with the Bruker database. In the Bruker database 17.4% of the isolates  
52 (29/167) were incorrectly identified as another species despite a score  $\geq 2.0$ .

53 **Conclusions:** The use of MALDI-TOF/MS in combination with a database developed with samples from  
54 18 *Achromobacter* species provides rapid and accurate identification. This tool could be used to help  
55 future clinical studies.

## 56 **Introduction**

57 *Achromobacter* spp. are increasingly reported in various infections in both immunocompromised and  
58 immunocompetent patients [1–3]. They are emerging pathogens in cystic fibrosis (CF) patients [4–6] but  
59 their role in the decline of respiratory function is not clear [6,7].

60 Over the last decade, the taxonomy of the genus *Achromobacter* has been revised.

61 Two current reference methods can be used to identify *Achromobacter* spp. to the species level: *nrdA*  
62 sequencing (765 bp fragment) or MultiLocus Sequence Typing (MLST/MLSA) (5 or 7 gene sequencing)  
63 [8-10]. The only available database, developed by Spilker and Lipuma  
64 (<https://pubmlst.org/achromobacter/>), provides online identification for 18 of the 21 described species: *A.*  
65 *xylosoxidans* [11], *A. denitrificans* [12], *A. ruhlandii*, *A. piechaudii* [13], *A. insolitus*, *A. spanius* [14], *A.*  
66 *marplatensis* [15], *A. insuavis*, *A. dolens*, *A. aegrifaciens*, *A. anxifer* [16], *A. animicus*, *A. mucicolens*, *A.*  
67 *pulmonis* [17], *A. agilis*, *A. pestifer*, *A. kerstersii* and *A. deleyi* [18]. These time-consuming techniques are  
68 not used for routine identification. Consequently *Achromobacter* spp. are mostly referred to as *A.*  
69 *xylosoxidans* in studies because conventional methods (biochemical tests, *rrs* sequencing, mass  
70 spectrometry (MS)) are not powerful enough to differentiate between species [10, 19-21]. The few studies  
71 performed with correct identification of the isolates have revealed that *A. xylosoxidans* was the most  
72 frequently encountered species and that the distribution of the other species was variable: from 6 to 14  
73 species among CF patients, and up to 12 species among non-CF patients [10, 22-25].

74 Given that the intrinsic anti-microbial resistance and probably also virulence properties differ from one  
75 species to another, a rapid and accurate method of identification is urgently needed [26].

76 For this purpose, mass spectrometry is a promising technique. However, the IVD-CE certified databases  
77 currently available in France (VITEK® MS bioMerieux and microflex LT Bruker) harbour only 2 (*A.*  
78 *xylosoxidans* and *A. denitrificans* for VITEK® MS V3.0) or 6 species (*A. xylosoxidans*, *A. denitrificans*,  
79 *A. ruhlandii*, *A. piechaudii*, *A. insolitus* and *A. spanius* for Bruker MS MBT-IVD-DB-7712 ).

80 To date, no study has evaluated the performance of species identification by MS on isolates  
81 identified by a reference method. Therefore, we aimed to generate a local database by MALDI-TOF/MS  
82 Bruker for routine identification of the 18 species of *Achromobacter* included in the pubMLST database

83 (*nrdA* analysis) when the study started and to compare its performance with that of the currently available  
84 Bruker default database.

85

## 86 **Methods**

### 87 **1) Bacterial isolates (supplementary data, Table S1 and S2 and S3)**

88 A total of 334 isolates belonging to 18 *Achromobacter* species were included. All had been previously  
89 identified to the species level by a reference method: sequencing 765 bp of the housekeeping gene *nrdA*  
90 [10] using the pubMLST database (<https://pubmlst.org/>) which contained 18 species. For each *nrdA*-765  
91 allele not matching an allele listed in the database, we had contacted T. Spilker to ensure a correct  
92 identification [4, 23]. The number of strains and alleles by species are listed in the Table S3 (total of 92  
93 alleles). Of 334 isolates, 192 were collected from the sputum of CF patients (96 from our CF centre  
94 (Dijon), 48 from 9 other French centres, 25 from Belgium, 9 from Denmark, and 14 from the USA), 71  
95 from various samples from non-CF patients and 53 from environmental samples (domestic, hospital,  
96 natural environments). Isolates of the various sources were included for both MSP (Main Spectra Profile)  
97 creation and database verification.

98 Type strains from the 18 *Achromobacter* species were also included.

### 99 **2) Generation of a local database (MSP creation) (supplementary data, Table S1)**

100 A total of 205 *Achromobacter* isolates, including the 18 type strains, were used to generate the local  
101 database (MSP creation): *A. aegrifaciens* (n=9), *A. agilis* (n=1), *A. animicus* (n=5), *A. anxifer* (n=1), *A.*  
102 *deleyi* (n=3), *A. denitrificans* (n=3), *A. dolens* (n=5), *A. insolitus* (n=8), *A. insuavis* (n=17), *A. kerstersii*  
103 (n=1), *A. marplatensis* (n=10), *A. mucicolens* (n=12), *A. pestifer* (n=1), *A. piechaudii* (n=1), *A. pulmonis*  
104 (n=4), *A. ruhlandii* (n=8), *A. spanius* (n=9), *A. xylosoxidans* (n=101), and 6 isolates belonging to a new  
105 *Achromobacter* species not characterized to date (novel species according to MLST analysis and  
106 confirmed by Dr P. Vandamme, personal communication) [4,23].

107 All isolates were cultivated on Mueller Hinton (MH) agar (Bio-Rad®) overnight at 37°C. MH is adapted  
108 to the culture of non-fastidious bacteria like *Achromobacter*. Extraction was performed according to  
109 Bruker instructions (MSP creation protocol V1.1). Briefly, one microliter loop of bacteria was suspended

110 in 300µl ultrapure water (HPLC-grade) then mixed with 900µl of 90% ethanol and centrifuged (12000 g  
111 for 2 min). The pellet was dried, resuspended in 50µl of 70% formic acid (Optima™, Fisher  
112 chemical)/acetonitrile (Carlo Erba Reagents) (V/V) and centrifuged again. The supernatant was used for  
113 further analysis.

114 Data acquisition was performed according to Bruker instructions (Maldi Biotyper protocol V.2.4). For  
115 each extract, the supernatant was spotted 8 times (1µl) on a disposable target, coated with 1µl HCCA  
116 matrix solution (α-cyano-4-hydroxy-cinnamic acid, Bruker Daltonics) and air -dried. The target was  
117 inserted in MALDI-TOF/MS Microflex LT (Bruker Daltonics), and FlexControl software (version 3.3,  
118 Bruker Daltonics) was used. Each spot was measured 3 times, resulting in 24 spectra by extract.

119 Each spectrum was generated with 240 laser shots (6 series of 40 laser shots) using linear positive mode  
120 with mass range  $m/z$  2,000 to 20,000, an acceleration voltage of 20kV in FlexControl software.  
121 Calibration was performed with the Bacterial Test Standard calibrant (BTS) (Bruker Daltonics,  
122 Germany).

123 The resulting 24 spectra were carefully inspected using flexAnalysis software (version 3.4 Bruker  
124 Daltonics), according to Bruker's recommendations. At least 20 spectra were selected to generate a single  
125 MSP onto the MALDI Biotyper 4.1 software (Bruker Daltonics) using the Biotyper MSP Creation  
126 Standard Method with default parameters (25% desired peak frequency minimum and 70 maximum  
127 desired peak number for the MSP). The MSPs were used to generate a new local database. After  
128 constitution of the novel *Achromobacter* database, each individually created MSP was analysed against  
129 the database itself to check if the best match was obtained with one MSP belonging to the same species.

130

### 131 **3) Evaluation for routine identification by direct colony transfer (supplementary data, Table** 132 **S1)**

133 Fifty isolates used for MSP creation (the 18 type strains and representative strains of all species) were  
134 selected to test the direct colony transfer method and assess the potential influence of culture media  
135 widely used for clinical samples analyses (sheep blood agar and Drigalski agar, bioMérieux). Isolates  
136 were tested in duplicate by direct colony transfer: colony fragments from overnight cultures at 37°C were

137 spotted onto the target and overlaid with 1µl of HCCA-matrix. MS analysis was performed as described  
138 above but with a single measure of each spot. The spectra obtained were compared to the newly generated  
139 database. The algorithm generated a log score value ranging from 0 to 3.0. We used the criteria provided  
140 by Bruker to interpret the score: a log score  $\geq 2.0$  indicated “high confidence identification” at the species  
141 level, a score value between 1.7 and 2.0 indicated “low confidence identification” and a score value  $< 1.7$   
142 “no organism identification possible”.

143

#### 144 **4) Accuracy of the newly generated database and comparison with default Bruker database** 145 **(supplementary data, Table S2)**

146 A total of 167 *Achromobacter* isolates (clinical and environmental) belonging to the 18 species were  
147 tested for database verification and comparison with the Bruker default database: *A. aegrifaciens* (n=7),  
148 *A. agilis* (n=1), *A. animicus* (n=2), *A. anxifer* (n=1), *A. deleyi* (n=3), *A. denitrificans* (n=3), *A. dolens*  
149 (n=6), *A. insolitus* (n=8), *A. insuavis* (n=18), *A. kerstersii* (n=1), *A. marplatensis* (n=5), *A. mucicolens*  
150 (n=14), *A. pestifer* (n=1), *A. piechaudii* (n=1), *A. pulmonis* (n=3), *A. ruhlandii* (n=13), *A. spanius* (n=6)  
151 and *A. xylooxidans* (n=74). Among these 167 isolates, 38 had already been used for the creation of the  
152 MSPs (Table S2, Figure S1). All isolates were cultivated (overnight at 37°C) on sheep blood agar, and 48  
153 of them also on MH and Drigalski agar (although MH and Drigalski are not approved media for the use of  
154 IVD-CE Bruker database).

155 Each isolate was tested in duplicate by direct colony transfer. Each spectrum was compared by the  
156 MALDI Biotyper software both to the default Bruker database (DB-7712 MSP) and to our database and  
157 the best matches and scores were gathered.

158

## 159 **Results**

### 160 **1) Verification of the newly generated database**

161 When analysing each individual MSP against the newly generated database, the best match corresponded  
162 to an MSP belonging to the same species and a score  $\geq 2.0$  in 94.6% (194/205) of cases (including the 6  
163 MSPs of the novel species 1).

164 The remaining cases involved the 5 species represented only once in the database (2.4%, 5/205), 2  
165 isolates not matching with a score  $\geq 2.0$ , and 4 isolates matching with another species (score  $\geq 2.0$ ),  
166 including one *A. ruhlandii* isolate that matched with *A. xylosoxidans*.

167

168 Identification performed by direct colony transfer of the 50 isolates led to 100% matching with their own  
169 corresponding MSP or another MSP from the same species. with an excellent degree (scores  $\geq 2.0$ ),  
170 indicating that direct transfer method is suitable. This identification occurred with colonies picked from  
171 sheep blood agar. Colonies picked from Drigalski agar yielded scores  $\geq 2.0$  for 78.0% (39/50) of the  
172 isolates.

173

## 174 **2) Accuracy of species identification by default Bruker database (Table 1)**

175 Briefly, the default Bruker database resulted in a correct identification (i.e. score  $\geq 2.0$  and concordance  
176 with *nrda*-sequencing) in only 50.9% of the isolates (85/167). Surprisingly, the species was misidentified  
177 in 17.4% of the isolates (29/167) despite high confidence scores ( $\geq 2.0$ ). All of these erroneously matched  
178 with *A. xylosoxidans* except for one strain of *A. piechaudii* that was identified as *A. spanius*. These errors  
179 mostly involved species not included in the database, with the exception of *A. ruhlandii* and *A.*  
180 *piechaudii*.

181 The other isolates led to scores  $< 2.0$  (25.1% (42/167) low confidence, and 6.6% (11/167) no match). The  
182 isolates not identified (score  $< 1.7$ ) belonged to species not found in the Bruker database.

183

## 184 **3) Accuracy of species identification with the local database (Table 1)**

185 Overall, 99.4% (166/167) of the isolates were correctly identified with our local database (i.e. score  $\geq 2.0$   
186 and concordance with *nrda*-sequencing). The only discrepancy observed involved a single isolate out of  
187 167 (0.6%), corresponding to one *A. ruhlandii* isolate for which the best match was obtained once with *A.*  
188 *xylosoxidans* (with *A. ruhlandii* as the second-best match with score  $\geq 2.0$ ). We therefore reiterated spots  
189 for all of the 13 *A. ruhlandii* isolates and observed that *A. xylosoxidans* erratically matched with high  
190 confidence scores as best or second-best match for 4 strains. For these 13 isolates, we also tested the



191 extraction method (used for database creation), which did not improve the results obtained. We did not  
192 detect any species-specific peaks in the spectra. In particular, none could be used to distinguish *A.*  
193 *xylosoxidans* from *A. ruhlandii*. The “10 % difference rule” proposed by several authors states that “any  
194 species scoring >10% below the top-scoring match may be excluded”.[27–29]. We observed that 11 out  
195 of 167 isolates failed this rule: 2 *A. mucicolens*, 1 *A. insolitus* and 2 *A. dolens* and interestingly 6 *A.*  
196 *ruhlandii* isolates.

197 Concerning the culture media used, high confidence scores were obtained for all isolates whether picked  
198 on MH or sheep blood agar. The scores were lower on Drigalski agar, and no score  $\geq 2.0$  was obtained for  
199 species other than *A. xylosoxidans*.

200

## 201 **Discussion**

202 In this study, we constructed the first MALDI-TOF database to include 18 species currently described  
203 within the genus *Achromobacter* using a wide collection of isolates identified with a reference method  
204 (*nrdA* sequencing). Because the pathogenicity of these bacteria remains controversial in CF patients, there  
205 is a strong need for developing rapid and accurate *Achromobacter* species identification methods that are  
206 currently not available for routine laboratory analysis. Concerning MALDI-TOF-MS, the commercially  
207 available libraries are incomplete: MALDI Biotyper® (Bruker) and VITEK® MS databases include  
208 respectively only 6 and 2 *Achromobacter* species.

209 Here we underscore the high number of erroneous identifications obtained with the Bruker database,  
210 despite high confidence scores, leading mostly to the misidentification of certain isolates as *A.*  
211 *xylosoxidans*, as already described [24]. One might assume that the spectra from isolates belonging to  
212 species not included in the database would not find a match but this this was not the case for isolates  
213 belonging to *A. insuavis*, *A. dolens* and *A. pestifer*. In these cases, it was not possible to determine a score  
214 cut-off for distinguishing erroneous and correct “*A. xylosoxidans*” identifications. Users should be aware  
215 of the risk of misidentification.

216 Moreover, the isolates belonging to species present in the Bruker database were not always correctly  
217 identified. Surprisingly none of the 13 *A. ruhlandii* isolates was correctly identified. This issue has  
218 already been pointed out by Rodrigues *et al.* in a study including 9 isolates of *A. ruhlandii* [21].  
219 Finally, 6.6% (11/167) of the isolates led to scores < 1.7, preventing genus identification. There may be  
220 several reasons for the issues with the Bruker database. Firstly, the number of isolates included in the  
221 database is limited and some species are absent, as already highlighted by Papalia *et al.* [24]. Secondly,  
222 with the exception of the type strains, the method of species identification of the isolates is not provided  
223 by the manufacturer, making it difficult to ascertain reliability. This also applies to VITEK® MS system.  
224 To overcome the weaknesses found in the existing databases, first we included only isolates identified by  
225 reference methods using the pubMLST database [10] and representatives of 18 species (corresponding to  
226 all the species that could be identified with this database at the beginning of the study. Second, the  
227 database was built up with a large number of MSPs (205) created with a wide variety of clinical and  
228 environmental isolates, either from our collection or from other collections in Denmark, Belgium, France,  
229 or the USA.

230 We built MSP dendrograms, but we noticed that the results depended on the parameters and the set of  
231 strains included for their construction (data not shown) despite the excellent results of species  
232 identification obtained with our database. This might be explained by the fact that Bruker algorithms for  
233 dendrogram construction and bacterial identification are not based on the same parameters.

234 With the use of our database, the rate of correct identifications jumped from 50.9% (85/167) (Bruker  
235 default database) to 99.4% (166/167) with high confidence scores (score  $\geq$  2.0) for all 167 isolates tested  
236 by direct colony transfer from sheep-blood agar. The use of Drigalski agar, which is not validated by  
237 Bruker, yielded lower scores, which could be explained by the drier texture of the colonies.

238 We recognize that our database has certain limitations.

239 Firstly, some species are rarely found in clinical samples [10, 22–25] and therefore not easily  
240 available from laboratory collections. As a result, few isolates were included in the database for some  
241 species: only 1 representative *A. agilis*, *A. anxifer*, *A. kerstersii*, *A. pestifer*, and *A. piechaudii*. In addition,  
242 accuracy was verified with less than 3 isolates for the 5 species listed above as well as for *A. animicus*. In

243 the future, additional strains belonging to these species or to novel species should be included when  
244 available to expand the database.

245 Secondly, the results were not satisfactory for some *A. ruhlandii* isolates. Indeed, the only  
246 incorrect species identification with our database (0.6%, 1/167) involved a single isolate of *A. ruhlandii*  
247 erroneously identified as *A. xylosoxidans*, and we observed this possible misidentification in the MSP  
248 evaluation step).-Gomila also noticed that “*A. xylosoxidans* and *A. ruhlandii* showed a close relationship  
249 by MALDI-TOF MS analysis” in a study including 10 *A. ruhlandii* isolates [30]. The inclusion of more  
250 strains of *A. ruhlandii* in our database might help to rectify this problem. On the contrary, for the 74 *A.*  
251 *xylosoxidans* isolates identified with our database, *A. ruhlandii* was never proposed with a high  
252 confidence score. Therefore, another method (*nrdA*-analysis or MLST) should be used when both *A.*  
253 *ruhlandii* and *A. xylosoxidans* are presented as the best and second-best match with high confidence  
254 scores.

255 In conclusion, we built a reliable database for the identification of clinical or environmental  
256 *Achromobacter*. This database has several valuable potential uses, including the enrichment of  
257 epidemiological data and the improvement of current understanding of the clinical impact of the various  
258 species of *Achromobacter*, which is particularly important for patients with CF.

### 259 **Transparency declaration**

260 The authors declare no conflict of interest.

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265

Species ( <i>nrDA</i> )	No. of strains	Bruker default database						New database					
		log score ≥ 2.0		1,7 ≤ log score < 2.0		log score < 1,7		log score ≥ 2.0		1,7 ≤ log score < 2.0		log score < 1,7	
		C	NC	C	NC	C	NC	C	NC	C	NC	C	NC
<i>A. aegrifaciens</i>	7	0	0	0	6 (85.7%)	0	1 (14.3%)	7 (100%)	0	0	0	0	0
<i>A. agilis</i>	1	0	0	0	1 (100%)	0	0	1 (100%)	0	0	0	0	0
<i>A. animicus</i>	2	0	0	0	0	0	2 (100%)	2 (100%)	0	0	0	0	0
<i>A. anxifer</i>	1	0	0	0	0	0	1 (100%)	1 (100%)	0	0	0	0	0
<i>A. deleyi</i>	3	0	0	0	1 (33.3%)	0	2 (66.7%)	3 (100%)	0	0	0	0	0
<i>A. denitrificans</i> *	3	2 (66.7%)	0	1 (33.3%)	0	0	0	3 (100%)	0	0	0	0	0
<i>A. dolens</i>	6	0	3 (50.0%)	0	3 (50.0%)	0	0	6 (100%)	0	0	0	0	0
<i>A. insolitus</i> *	8	5 (62.5%)	0	3 (37,5%)	0	0	0	8 (100%)	0	0	0	0	0
<i>A. insuavis</i>	18	0	17 (94.4%)	0	1 (5.6%)	0	0	18 (100%)	0	0	0	0	0
<i>A. kerstersii</i>	1	0	0	0	1 (100%)	0	0	1 (100%)	0	0	0	0	0
<i>A. marplatensis</i>	5	0	0	0	3 (60.0%)	0	2 (40.0%)	5 (100%)	0	0	0	0	0
<i>A. mucicolens</i>	14	0	0	0	12 (85.7%)	0	2 (14.3%)	14 (100%)	0	0	0	0	0
<i>A. pestifer</i>	1	0	1 (100%)	0	0	0	0	1 (100%)	0	0	0	0	0
<i>A. piechaudii</i> *	1	0	1 (100%)	0	0	0	0	1 (100%)	0	0	0	0	0
<i>A. pulmonis</i>	3	0	0	0	2 (66.7%)	0	1 (33.3%)	3 (100%)	0	0	0	0	0
<i>A. ruhlandii</i> *	13	0	7 (53.8%)	3 (23.1%)	3 (23.1%)	0	0	12 (92.3%)	1 (7.7%)	0	0	0	0
<i>A. spanius</i> *	6	4 (66.7%)	0	2 (33.3%)	0	0	0	6 (100%)	0	0	0	0	0
<i>A. xylosoxidans</i> *	74	74 (100%)	0	0	0	0	0	74 (100%)	0	0	0	0	0
Total	167	85 (50.9%)	29 (17.3%)	9 (5.4%)	33 (19.8%)	0	11 (6.6%)	166 (99.4%)	1 (0.6%)	0	0	0	0

267

268 **Table 1 : Comparison of the results of species identification of 167 isolates by Bruker default database and the newly generated database.**

269 C: result of identification concordant with *nrDA* sequencing

270 NC: result of identification non-concordant with *nrDA* sequencing

271 \* Species included in Bruker default database.

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362

Species ( <i>nrDA</i> )	No. of strains	Bruker default database						New database	
		log score $\geq 2.0$		1,7 $\leq$ log score $< 2.0$		log score $< 1,7$		log score $\geq 2.0$	
		C	NC	C	NC	C	NC	C	NC
<i>A. aegrifaciens</i>	7	0	0	0	6 (85.7%)	0	1 (14.3%)	7 (100%)	0
<i>A. agilis</i>	1	0	0	0	1 (100%)	0	0	1 (100%)	0
<i>A. animicus</i>	2	0	0	0	0	0	2 (100%)	2 (100%)	0
<i>A. anxifer</i>	1	0	0	0	0	0	1 (100%)	1 (100%)	0
<i>A. deleyi</i>	3	0	0	0	1 (33.3%)	0	2 (66.7%)	3 (100%)	0
<i>A. denitrificans</i> *	3	2 (66.7%)	0	1 (33.3%)	0	0	0	3 (100%)	0
<i>A. dolens</i>	6	0	3 (50.0%)	0	3 (50.0%)	0	0	6 (100%)	0
<i>A. insolitus</i> *	8	5 (62.5%)	0	3 (37,5%)	0	0	0	8 (100%)	0
<i>A. insuavis</i>	18	0	17 (94.4%)	0	1 (5.6%)	0	0	18 (100%)	0
<i>A. kerstersii</i>	1	0	0	0	1 (100%)	0	0	1 (100%)	0
<i>A. marplatensis</i>	5	0	0	0	3 (60.0%)	0	2 (40.0%)	5 (100%)	0
<i>A. mucicolens</i>	14	0	0	0	12 (85.7%)	0	2 (14.3%)	14 (100%)	0
<i>A. pestifer</i>	1	0	1 (100%)	0	0	0	0	1 (100%)	0
<i>A. piechaudii</i> *	1	0	1 (100%)	0	0	0	0	1 (100%)	0
<i>A. pulmonis</i>	3	0	0	0	2 (66.7%)	0	1 (33.3%)	3 (100%)	0
<i>A. ruhlandii</i> *	13	0	7 (53.8%)	3 (23.1%)	3 (23.1%)	0	0	12 (92.3%)	1 (7.7%)
<i>A. spanius</i> *	6	4 (66.7%)	0	2 (33.3%)	0	0	0	6 (100%)	0
<i>A. xylosoxidans</i> *	74	74 (100%)	0	0	0	0	0	74 (100%)	0
Total	167	85 (50.9%)	29 (17.3%)	9 (5.4%)	33 (19.8%)	0	11 (6.6%)	166 (99.4%)	1 (0.6%)

**Table 1 :** Comparison of the results of species identification of 167 isolates by Bruker default database and the newly generated database.

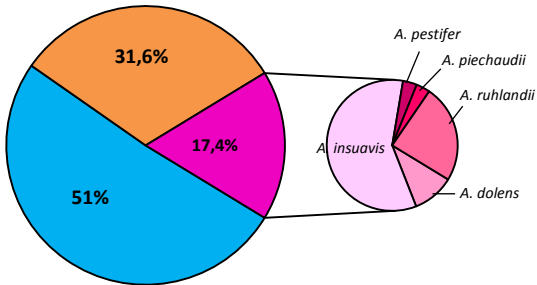
C: result of identification concordant with *nrDA* sequencing

NC: result of identification non-concordant with *nrDA* sequencing

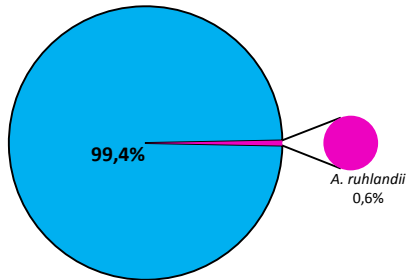
\* Species included in Bruker default database.



Identification by Bruker default database



Identification by our newly generated database



■ Misidentification with score  $\geq 2$

■ Score  $< 2$

■ Concordant identification with score  $\geq 2$