

Identification of Bacteria Directly from Positive Blood Culture Samples by DNA  
Pyrosequencing of *16S rRNA*

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## ABSTRACT

1           Rapid identification of causative bacteria in patients with sepsis can contribute to  
2 appropriate selection of antibiotics and improvement of patients' prognosis. Genotypic  
3 identification is an emerging technology that may provide an alternative method to, or  
4 complement, established phenotypic identification procedures. We evaluated a rapid protocol  
5 of bacterial identification based on polymerase chain reaction and pyrosequencing of V1 and  
6 V3 gene of *16S rRNA* using DNA directly from positive blood culture samples. One hundred  
7 and two culture positive blood culture bottles from 68 patients were randomly selected and  
8 the contained bacteria were identified by phenotyping and pyrosequencing. Pyrosequencing  
9 identification displayed 84.3% and 64.7% concordance with phenotypical identification at the  
10 genus and species levels, respectively. In the monomicrobial samples, the concordance at the  
11 genus level was 87.0%. Pyrosequencing identified one isolate in 60% of polymicrobial  
12 samples that were confirmed by culture analysis. Of the pyrosequencing-identified samples,  
13 the result of V1 and V3 were consistent in 55.7% and the other samples were identified based  
14 on the results of V1 (12.5%) or V3 (31.8%). One isolate was erroneously identified by  
15 pyrosequencing due to a highly similar sequence with another isolate. Pyrosequencing  
16 identified one isolate that was not detected by phenotyping. The process of pyrosequencing  
17 identification can be completed within approximately 4 h. The information provided by  
18 DNA-pyrosequencing identification of microorganism isolates in positive blood culture  
19 bottles is accurate and could provide a rapid and useful tool in standard laboratory practice.

20

21 Key words: genetic identification, pyrosequence, *16S rRNA*

22

## 23 INTRODUCTION

24

25           Blood stream infections such as severe sepsis and septic shock result in high  
26 mortality. Detection and identification of causative microorganisms of sepsis are crucial for  
27 selection of the appropriate antimicrobial agents. Blood culture is an important method for  
28 the growth and subsequent identification of causative microorganisms, and diagnostic  
29 laboratories are required to detect such microorganisms as rapidly as possible. Accurate  
30 identification of bacterial isolates is also an essential task of the clinical microbiology  
31 laboratory. While traditional phenotypic identification is universally used in clinical  
32 laboratories, this method has some disadvantages. For example, it is time consuming,  
33 sometimes difficult, and does not always accurately identify target microorganisms. In  
34 addition, interpretation of the results obtained using phenotypic methods can involve  
35 substantial subjective judgment (Stager & Davis, 1992).

36           Genotypic identification of microorganisms is an emerging technology that may  
37 provide an alternative or a complementary method to established phenotypic identification  
38 procedures. Sequence analysis of the *16S rRNA* gene is a widely accepted tool for molecular  
39 identification of bacteria (Kolbert & Persing, 1999; Patel, 2001; Woese, 1987). Bacterial *16S*  
40 *rRNA* genes consist of eight highly conserved and nine variable regions (Woese, 1987). V1  
41 and V3 are two distinct variable regions included in the *16S rRNA* gene that have been used  
42 as targets for a sequencing-based identification assay (Luna *et al.*, 2007). This assay  
43 capitalizes on the highly conserved nature of *16S rRNA* genes by positioning amplification  
44 and sequencing primers in the conserved regions that flank the variable regions (specifically  
45 V1 and V3), thereby allowing primers to theoretically amplify most bacterial pathogens.  
46 Public databases such as GenBank, the Nucleotide Sequence Database at the European  
47 Molecular Biology Laboratory (EMBL-Bank), the DNA Data Bank of Japan (DDBJ) and the  
48 Ribosomal Database Project II (RDP II) contain a vast number of bacterial *16S rRNA*

49 sequences, allowing for rapid analysis and providing phylogenetically meaningful  
50 information (Bosshard *et al.*, 2006).

51 Pyrosequencing is a DNA sequencing technique that is based on the detection of  
52 pyrophosphate that is released during DNA synthesis and was introduced as a rapid  
53 alternative to traditional Sanger DNA sequencing (Ronaghi *et al.*, 1996). The DNA base  
54 sequence is determined by measuring the strength of visible light that is generated in  
55 proportion to the number of incorporated nucleotides in a cascade of enzymatic reactions  
56 (Ronaghi, 2001). The main advantage of pyrosequencing is its rapidity and lower price  
57 compared to conventional sequencing. Although the length of the sequence that can be  
58 obtained by pyrosequencing is fairly short and limited to about 30-60 bases, carefully  
59 designed applications can provide information that is sufficient for the differentiation of gene  
60 sequences. Pyrosequencing has already been applied to identification of bacteria in the field  
61 of microbiology (Jonasson *et al.*, 2002; Luna *et al.*, 2007; Ronaghi & Elahi, 2002). It has also  
62 been predicted that pyrosequencing of the *16S rRNA* gene will be a useful tool for the  
63 identification of bacteria, and may function as a “molecular gram stain” (Jordan *et al.*, 2005).  
64 For patients with sepsis, the rapid identification of causative bacteria is important, however,  
65 the conventional phenotyping-based identification requires an extra day after blood culture  
66 become positive. Therefore, the rapidity of pyrosequencing-based identification is an  
67 attractive advantage for diagnosis. In fact, Jordan *et al.* reported that the combination  
68 methods of real-time polymerase chain reaction (PCR) and pyrosequencing rapidly identified  
69 bacteria from positive blood culture samples and provided highly-concordant results with the  
70 phenotypic identification (Jordan *et al.*, 2009).

71 To rapidly identify clinical isolates from positive blood culture samples, we  
72 evaluated a rapid protocol for microorganism identification using PCR and pyrosequencing  
73 of *16S rRNA*. Using clinical samples from our hospital, we compared our bacterial  
74 identification protocol with conventional culture identification.

75

## 76 METHODS

77

### 78 Sample collection

79           This study was performed at the Nagasaki University Hospital that is a tertiary  
80 hospital with about 850 beds and approved by the ethics committee of Nagasaki University  
81 Hospital. The positive blood culture samples were randomly selected from the blood culture  
82 bottles during 2010 that were submitted for the usual microbiological testing from both  
83 pediatrics and adults. The blood sampling was performed according to the recommended  
84 methods in our hospital and 5-10 mL of blood were collected into the each bottle. Bottles  
85 containing samples from the same patient but at different time points were excluded.

86

### 87 Blood culture and phenotypical identification

88           Blood samples that were collected in BacT/ALERT FA or BacT/ALERT FN bottles  
89 (bioMerieux, Hazelwood, MO) at the Nagasaki University Hospital were cultured using  
90 BacT/ALERT 3D (bioMerieux, Hazelwood, MO), which is an automated microbial detection  
91 system that displays a positive result if microbial growth is detected by a fluorescent sensor.  
92 Each bottle was removed from the blood culture instrument within 12 h after the bottle went  
93 positive and > 1 mL samples were immediately extracted from the bottle. The sample was  
94 gram stained and subcultured on the appropriate agar-based culture plates. All samples were  
95 identified according to standard biochemical identification methods using the VITEK 2  
96 system (bioMerieux, Hazelwood, MO) or the Phoenix100 system (Becton Dickinson,  
97 Franklin Lakes, NJ).

98

### 99 DNA extraction and amplification

100           Bacterial DNA was extracted directly from 1mL of the blood culture fluid using the

101 BiOstic bacteremia DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) according to  
102 the manufacturer's instructions. Chromosomal DNA was eluted in a final volume of 50  $\mu$ L of  
103 elution buffer. The V1, amplicon size 115bp, and V3, amplicon size 81bp, regions of *16S*  
104 *rRNA* genes were amplified according to a previously published method (Luna *et al.*, 2007).  
105 Nucleotide positions refer to positions in the *Escherichia coli 16S rRNA* gene. The Bio-pBR5  
106 (5'-biotin-GAAGAGTTTGATCATGGCTCAG-3') and pBR-V1  
107 (5'-TTACTCACCCGTCCGCCACT-3') primers were used for V1 amplification, and the  
108 Bio-B-V3 (5'-biotin-ACGACAGCCATGCAGCACCT-3') and pJBS.V3  
109 (5'-GCAACGCGAAGAACCTTACC-3') primers were used for V3 amplification. Each 50  
110  $\mu$ L reaction mixture contained 25  $\mu$ L of Ampdirect (Shimadzu Co., Kyoto, Japan), 0.2 M of  
111 each primer, 1.25 U AmpliTaq Gold DNA polymerase LD (Life Technologies, Carlsbad, CA)  
112 and 5  $\mu$ L of DNA template. PCR was performed using the GeneAmp PCR system 9700 (Life  
113 Technologies, Carlsbad, CA) with the following cycling parameters: 10 min at 95°C, 35  
114 cycles of 95°C for 40 s, 55°C for 40 s and 72°C for 60 s, followed by a single cycle of 72°C  
115 for 60 s. The DNA extracted from the clinical isolates including *Staphylococcus aureus*,  
116 *Bacillus cereus* and *Escherichia coli* and sterile water were used as positive controls and a  
117 negative control for PCR, respectively. Each PCR product was verified by agarose gel  
118 electrophoresis. The samples without single band were amplified after 10- or 100-fold  
119 dilution and reconfirmed by agarose gel analysis.

120

#### 121 DNA pyrosequencing

122 The amplified V1 and V3 products were prepared for pyrosequencing by using the  
123 recommended protocol for the vacuum prep tool (Qiagen, Valencia, CA). For the preparation  
124 of each reaction, 40  $\mu$ L of the biotinylated PCR product was used. To prepare the sequencing  
125 plate, purified PCR products were resuspended in 43  $\mu$ L of binding buffer and 3  $\mu$ L of  
126 streptavidin beads. Double-stranded DNA was then denatured to single-stranded DNA using a

127 0.2 M NaOH. Subsequently, single-stranded DNA was resuspended in 40 µL of annealing  
128 buffer with 0.3 µM sequencing primer and then annealed to the sequencing primer at 80°C  
129 for 2 min. The primers pBR-V1 and pJBS.V3 as described above were used as DNA  
130 sequencing primers for the V1 and V3 regions, respectively. Pyrosequencing was performed  
131 on the PyroMark ID instrument (Qiagen, Valencia, CA) with 8 cycles of a repetitive ACTG  
132 dispensation. Sequence homology of PCR products was compared using the DDBJ search  
133 program ([www.ddbj.nig.ac.jp](http://www.ddbj.nig.ac.jp)) and a strain with >99% sequence homology was considered as  
134 an isolated strain.

135

## 136 RESULTS

137

### 138 Culture results

139 In this study, 102 samples collected from 68 patients were cultured and 112 bacteria  
140 and 1 fungus (*Candida albicans*) were isolated. Two types of microorganisms were isolated  
141 in each of 10 (9.8%) samples from seven cases. The cultured bacteria included 15 genera and  
142 28 species. One isolate was not identified and is referred to as an anaerobic gram-positive  
143 rod.

144

### 145 Detection and identification of microorganisms by DNA-pyrosequencing

146 All 102 samples were successfully amplified by PCR targeted on V1 or V3. Four  
147 samples required dilution for amplification of the products because of inhibition. DNA  
148 pyrosequencing-based identification was then performed using these PCR products. From the  
149 102 samples, 88 (86.3%) and 68 (66.7%) strains were detected to the genus and species level,  
150 respectively, by DNA pyrosequencing. These bacteria were separated into 16 genera and 19  
151 species. The strains included 41 gram-positive cocci, 9 gram-positive bacilli, 34  
152 gram-negative bacilli and 4 anaerobic organisms. Of 68 cases, isolates from 61 (89.7%) and

153 49 (72.1%) cases were detected to the genus and species level, respectively.

154

155 Culture results and pyrosequencing identification of V1 and V3 gene

156 The pyrosequencing results corresponding to each culture-based organism were  
157 analyzed. In the monomicrobial samples (Table 1), 21 strains were completely agreed with  
158 the culture-based identification and both the two pyrosequencing identifications at species  
159 level. These completely-concordant strains were observed in *Staphylococcus aureus*, *S.*  
160 *epidermidis*, *Corynebacterium striatum*, *Escherichia coli* and *Pseudomonas aeruginosa*. The  
161 other concordant isolates at species level were dependent on either target (V1, 20 isolates; V3,  
162 22 isolates). In two isolates, pyrosequencing presented different strains from the  
163 culture-based identification. One of these resulted from the consistent identification of V1  
164 and V3 and the other from the V3 sequencing.

165 In the polymicrobial samples (Table 2), pyrosequencing failed to completely identify  
166 all of the bacteria that were identified by the culture method. However, one organism was  
167 identified in six (60%) of these samples and three of the six strains were identified to species  
168 level by pyrosequencing. One was based on both results of V1 and V3 sequence and the  
169 others were based on V1 sequencing.

170 However, in some bacteria, each target of *16S rRNA* could not successfully identify  
171 the bacterium at both species and genus levels. Genus *Enterobacter*, *Bacteroides fragilis*,  
172 *Fusobacterium nucleatum*, *Bifidobacterium scardovii* were not detected by the sequencing of  
173 V1. In contrast, the pyrosequencing of V3 failed to detect *Citrobacter freundii* and Genus  
174 *Clostridium*.

175

176 Concordance rate of sequence-based identification

177 The percentage of concordance between culture-based and pyrosequence-based  
178 identification was calculated (Table 3). Of the 92 monomicrobial samples identified by



179 culture, 80 (87.0%) samples at genus level and 63 (68.5%) samples at species level were  
180 concordant with pyrosequence-based identifications. Two (2.2%) samples showed discordant  
181 results and 10 (10.9%) samples were unidentified by pyrosequencing.

182 Of the 10 polymicrobial samples confirmed by culture, pyrosequencing identified  
183 one microorganism in 6 samples at genus level with concordance and in 3 samples at species  
184 level. Pyrosequencing did not detect two or more microorganisms in all samples.

185 The overall agreement between culture- and pyrosequence-based identification was  
186 84.3% (86/102) at genus level and 64.7% (66/102) at species level.

187

188 Analysis of discordant results

189 Two samples displayed discordant identification between DNA pyrosequencing and  
190 phenotyping. In one sample, the isolate was determined as *Staphylococcus epidermidis* by  
191 pyrosequencing. However, the characteristics of this isolate were inconsistent with  
192 biochemical data of *S. epidermidis* and the Phoenix100 system and VITEK2 system analysis  
193 identified this isolate as *Staphylococcus capitis* with 99% probability. The sequence  
194 homology of the *16S rRNA* of *S. epidermidis* and *S. capitis* is 99%. The pyrosequencing  
195 result was interpreted as a false positive result.

196 In the other sample, the isolate was identified as *Bifidobacterium scardovii* by  
197 pyrosequencing, but the conventional culture method identified it simply as an anaerobic  
198 gram-positive rod that could not be further classified because of poor data regarding  
199 morphological and biochemical characteristics. This isolate was ultimately determined as *B.*  
200 *scardovii* after confirming the reproducibility.

201

202 DISCUSSION

203

204 Rapid identification of causative bacteria in patients with sepsis can lead to the

205 appropriate selection of antibiotics (Barenfanger *et al.*, 1999) and the improvement of  
206 prognosis (Barenfanger *et al.*, 2001). Bacterial identification based on genetic methods can  
207 provide information that is useful for the selection of targeted antibiotics.

208         The overall isolate information obtained by pyrosequencing agreed with the  
209 information obtained using the culture method for 84.3% and 64.7% of isolates at the genus  
210 and species level, respectively. A previous report of DNA pyrosequencing identification that  
211 used pure-cultured isolates reported approximately 90% agreement between the isolates  
212 identified by the two methods (Luna *et al.*, 2007). Considering that DNA was extracted  
213 directly from blood culture bottle fluids, we believe that the concordance between the two  
214 methods that we observed is reasonable. In addition, pyrosequencing resulted in only one  
215 error in sample identification, which was due to very high sequence similarity between two  
216 bacteria, implying that DNA-pyrosequencing is a very accurate method for identification of  
217 bacteria. These results suggest that DNA-pyrosequencing identification of bacteria in positive  
218 blood culture samples will be useful for evaluation of clinical samples and can contribute to  
219 appropriate antimicrobial treatment and benefit patient outcome.

220         Previously, Jordan *et al.* reported a highly-accurate pyrosequencing identification  
221 from the positive blood culture bottles (Jordan *et al.*, 2009). In Jordan's report, the *23S rRNA*  
222 gene was used as the targets to improve the identification efficiency of some specific bacteria  
223 such as *Enterobacteriae* and *Streptococcus* species, and the agreement between  
224 pyrosequencing- and culture-based identification reached 97.8%. In the present study, the  
225 concordant rate was lower than Jordan's report. This was partly because a larger number of  
226 polymicrobial samples was included than Jordan's report. Furthermore, the relatively large  
227 number of undetected specific strains such as Genus *Enterobacter* could also decrease the  
228 concordant rate of this study.

229         In this study, the sequences of V1 and V3 represented similar results in many  
230 samples but also showed different characteristics in some specific bacteria. V1 can effectively

231 classify genus *Enterococcus* into *E. faecalis* or *E. faecium* and V3 can have advantages of  
232 detecting *S. epidermidis* and *E. coli*. These suggested that the sequencing V1 and V3  
233 improved the accuracy of diagnosis. However, the best combination of variable regions of  
234 *16S rRNA* for diagnosis has been a controversial issue (Sundquist *et al.*, 2007; Wang *et al.*,  
235 2007).

236 Conventional biochemical testing, especially for difficult-to-identify pathogens, may  
237 result in incorrect pathogen identification, resulting in inconsistent information for the  
238 physician (Downes *et al.*, 1998; Stager & Davis, 1992). Molecular methods provide novel  
239 strategies for bacterial pathogen identification (Tang *et al.*, 1998). *16S rRNA* sequencing was  
240 previously reported to detect relevant isolates of nonfermenting gram-negative bacilli at high  
241 rates compared to phenotypic identification. The reason for the low rate of phenotypic  
242 identification was that nearly half of the isolates that corresponded to species based on  
243 sequencing data were not included in the databases of conventional phenotypic identification  
244 systems (Bosshard *et al.*, 2006). Molecular methods are considered useful for identification  
245 of gram-positive bacteria or anaerobes as well as of gram-negative bacteria. In this study, *B.*  
246 *scardovii* was identified by the genetic method but not by the usual laboratory procedures.  
247 Therefore, the genetic method described in this study may complement current methods of  
248 phenotypical identification.

249 Although the method described in this study is considered to be a useful and  
250 convenient procedure for rapid identification of microorganisms, it also has some limitations  
251 in terms of efficacy of identification. First, pyrosequencing can fail to separate distinct  
252 bacteria which have similar sequences because it only reads short sequence lengths. The  
253 genera *Aeromonas*, *Bacillus* and *Staphylococcus* are typical genera which have similar  
254 sequences in the target gene in each genus. Therefore, organisms which belong to these  
255 genera were not effectively identified at the species level, but showed good agreement with  
256 culture results at the genus level. Other specific sequencing targets will be required to

257 identify the correct species of these bacteria. However, with the exception of *Staphylococci*,  
258 these genera are rarely isolated and their antibiotic resistance has not become problematic.  
259 Therefore, it is considered that genetic methods to identify these bacteria at the species level  
260 may not be necessary. Second, in polymicrobial infections, pyrosequencing may not identify  
261 all of the bacteria. Thus, when a sample for pyrosequencing contains polymicrobial genes, the  
262 result obtained from sequencing can consist of a mix of sequences from those organisms.  
263 Therefore, pyrosequencing may not effectively detect organisms in patients with  
264 polymicrobial infection. Among bacteria that were undetectable by pyrosequencing, some  
265 species such as *E. cloacae*, *E. faecalis*, *B. fragilis* and *B. thuringiensis* were commonly  
266 observed, and the samples that included these isolates were often polymicrobial. Most of the  
267 bacteria that were not detected by pyrosequencing in this study are commonly known as  
268 causative pathogens of intra-abdominal and urinary tract infections in which polymicrobial  
269 infections are often observed (Reuben *et al.*, 1989).

270 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry  
271 (MALDI-TOF MS) has been used as an accurate identification tool with fast and  
272 cost-effective benefits. Identification directly from positive blood cultures by using  
273 MALDI-TOF MS has been also attempted and 74.3 – 98.0 % of bacteria were correctly  
274 identified to the species level (Christner *et al.*, 2010; Wimmer *et al.*, 2012; Wuppenhorst *et al.*,  
275 2012). However, some bacteria including *E. coli* and *Shigella* spp. are known as  
276 indistinguishable bacteria by MALDI-TOF MS. Especially, *Streptococcus* spp. is not reliably  
277 identified to the species level in both MALDI-TOF MS and *16S RNA* pyrosequencing.  
278 Therefore, these rapid protocols require the additional procedures to identify these bacteria  
279 correctly.

280 The process of pyrosequencing identification of bacteria that was used in this study  
281 including sample preparation, the sequencing reaction and analysis of the results, can be  
282 completed within approximately 4 h. Repeated sequencing from the same sample bottle

283 provided consistent results. This method would therefore be relatively easy to fit into a  
284 standard routine work and obtaining information regarding the isolate within a day would be  
285 of great help in improving the outcome of sepsis.

286

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290 Technology, and a grant from the Global Centers of Excellence Program, Nagasaki  
291 University.

292

#### 293 CONFLICT OF INTERESTS

294 The authors declare that they have no conflict of interests.

295

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353  
354

355 Table 1. Phenotypical identification and the distribution of pyrosequencing identification results in monomicrobial samples

356

Phenotypical identification		No. of concordance/discordance with pyrosequencing identification					
Strains	No. of isolates	Concordant				Discordant	Undetected
		Species level			Genus level		
		V1+V3 <sup>a</sup>	V1 <sup>b</sup>	V3 <sup>c</sup>			
Gram-positive cocci							
<i>Enterococcus faecalis</i>	2	0	2	0	0	0	0
<i>Enterococcus faecium</i>	3	0	2	0	0	0	1
<i>Staphylococcus aureus</i>	15	9	4	2	0	0	0
<i>Staphylococcus capitis</i>	2	0	0	0	0	1 <sup>d</sup>	1
<i>Staphylococcus epidermidis</i>	9	4	0	3	2	0	0
<i>Staphylococcus haemolyticus</i>	2	0	1	0	1	0	0
<i>Staphylococcus hominis</i>	1	0	0	1	0	0	0
<i>Staphylococcus schleiferi</i>	1	0	0	0	1	0	0
<i>Staphylococcus simulans</i>	2	0	0	0	2	0	0
<i>Staphylococcus agalactiae</i>	3	0	2	1	0	0	0
Gram-positive bacilli							
<i>Bacillus cereus</i>	3	0	1	0	2	0	0
<i>Bacillus thuringiensis</i>	1	0	0	0	1	0	0
<i>Corynebacterium striatum</i>	2	2	0	0	0	0	0
Gram-negative bacilli							
<i>Aeromonas hydrophila</i>	1	0	0	0	1	0	0
<i>Aeromonas sobria</i>	3	0	0	0	3	0	0



<i>Citrobacter freundii</i>	2	0	2	0	0	0	0
<i>Enterobacter aerogenes</i>	1	0	0	0	0	0	1
<i>Enterobacter cloacae</i>	7	0	0	0	3	0	4
<i>Escherichia coli</i>	16	3	2	10	0	0	1
<i>Haemophilus influenzae</i>	1	0	0	1	0	0	0
<i>Klebsiella oxytoca</i>	1	0	1	0	0	0	0
<i>Klebsiella pneumoniae</i>	6	0	2	2	0	0	2
<i>Pseudomonas aeruginosa</i>	3	3	0	0	0	0	0
<i>Pseudomonas putida</i>	1	0	1	0	0	0	0
Others (anaerobes)							
<i>Bacteroides fragilis</i>	1	0	0	1	0	0	0
<i>Fusobacterium nucleatum</i>	1	0	0	1	0	0	0
Genus <i>Veillonella</i>	1	0	0	0	1	0	0
Anaerobic gram-positive rod	1	0	0	0	0	1 <sup>e</sup>	0
Total	92	21	20	22	17	2	10

357 <sup>a</sup> Consistent results of V1 and V3 sequence. <sup>b</sup> Identification by V1 sequence. <sup>c</sup> Identification by V3 sequence. <sup>d</sup> This isolate was misidentified as  
358 *Staphylococcus epidermidis* by both V1 and V3 sequencing. <sup>e</sup> This isolate was identified as *Bifidobacterium scardovii* by V3 sequencing.

359 Table 2. Pyrosequencing identification in polymicrobial samples

360

Phenotypical identification		Pyrosequencing identification			
Strains	No. of samples	Strains	Concordant <sup>a</sup> (no.)		Undetected (no.)
			Species level	Genus level	
<i>Bacillus thuringiensis</i> / <i>Staphylococcus saprophyticus</i>	2	Genus <i>Bacillus</i>	0	2 <sup>b</sup>	0
<i>Bacteroides fragilis</i> / <i>Clostridium clostridioforme</i>	1	Genus <i>Clostridium</i>	0	1 <sup>c</sup>	0
<i>Enterobacter cloacae</i> / <i>Enterococcus faecalis</i>	2	undetected	0	0	2
<i>Enterobacter cloacae</i> / Genus <i>Bacteroides</i>	1	undetected	0	0	1
<i>Enterobacter cloacae</i> / <i>Staphylococcus aureus</i>	1	<i>Staphylococcus aureus</i>	1 <sup>d</sup>	0	0
<i>Enterococcus faecium</i> / <i>Staphylococcus heamolyticus</i>	2	<i>Enterococcus faecium</i>	2 <sup>e</sup>	0	0
<i>Staphylococcus aureus</i> / <i>Candida albicans</i>	1	undetected	0	0	1

361 <sup>a</sup> When at least one isolate was the same result, the sample was considered concordant.

362 <sup>b</sup> Identification based on V3 sequence. <sup>c</sup> Identification based on V1 sequence.

363 <sup>d</sup> Identification based on both V1 and V3 sequence. <sup>e</sup> Identification based on V1 sequence.

364 Table 3. Summary of the concordance of DNA pyrosequencing identification with  
 365 phenotypical identification  
 366

Results of pyrosequencing identification	Phenotypical identification, n (%)		
	Monomicrobial (n = 92)	Polymicrobial* (n = 10)	All (n = 102)
Genus level			
Detected			
Concordant	80 (87.0)	6 (60.0)	86 (84.3)
Discordant	2 (2.2)	0 (0.0)	2 (2.0)
Undetected	10 (10.9)	4 (40.0)	14 (13.7)
Species level			
Detected			
Concordant	63 (68.5)	3 (30.0)	66 (64.7)
Discordant	2 (2.2)	0 (0.0)	2 (2.0)
Undetected	27 (29.3)	7 (70.0)	34 (33.3)

367  
 368 \* When at least one isolate was the same result, the sample was considered concordant.

369 FIGURE LEGENDS

370

371 Figure 1. A representative pyrogram of V1 and V3 gene.

372 A representative pyrogram of targeted V1 (A) and V3 (B) gene. The sequence results were  
373 shown at the bottom of each pyrogram. This sample was identified as *P. aeruginosa* after  
374 homology search.

