

## **SUPPLEMENTAL MATERIALS AND METHODS**

**Performance of MALDI Biotyper system.** All the isolates were incubated on Trypticase soy agar with 5% sheep blood (BAP) (Becton-Dickinson Microbiology Systems, Sparks, MD, USA) and incubated for 24 hours at 37°C. Two to three colonies were transferred to 50 µl of 70% formic acid. After incubation for 30 seconds, 50 µl of acetonitrile (Sigma-Aldrich) was added. The suspension was then centrifuged at 13,000 rpm for 2 min. A volume of 1.0 µl of the supernatant was applied to a 96-spot polished steel target (Bruker Daltonik GmbH) plate and dried. A saturated solution of 1.0 µl of MALDI matrix (HCCA; Bruker Daltonik GmbH) was applied to each sample and dried. Measurements were performed with the MALDI Biotyper system using the FlexControl™ software with the Compass Flex Series version 1.3 software and a 60Hz nitrogen laser (337 nm wave length). Spectra were collected in the linear positive mode in a mass range covering 1,960 to 20,132 m/z. Spectra ranging from the mass-to-charge ratio (m/z) 2,000 to 20,000 were analyzed using the MALDI Biotyper system automation control and the Bruker BioTyper 3.1 software and library (DB 5627 with 5,627 entries). Identification scores of  $\geq 2.000$  indicated species-level identification, scores of 1.700 to 1.999 indicated genus-level identification, and scores of  $< 1.700$  indicated no reliable identification. The nine control strains, *A. aquariorum* MDC47<sup>T</sup>, *A. hydrophila* subsp. *dhakensis* LMG 19562, *A. hydrophila* ATCC 7966<sup>T</sup>, *A. veronii* biovar *sobria* ATCC 9071<sup>T</sup>, *A. caviae* ATCC 13136<sup>T</sup>, *A. hydrophila* BCRC 16704, *A. hydrophila* BCRC 13881, BCRC 17768 (*A. hydrophila* subsp. *ranae* Huys et al., 2003), and *A. bestiarum* ATCC 13444, were obtained from the Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan.

### **Species identification by *rpoB* sequencing**

As previously described, identification of *Aeromonas* species was based on the sequence analysis of the partial *rpoB* gene by PCR with the primers: PasrpoB-L (5'-GCAGTGAAAGARTTCTTTGGTTC-3') and RpoB-R (5'-GTTGCATGTTNGNACCCAT-3') (1). The sequences of the 560 base-pair amplified DNA products were compared with reference sequences available in the GenBank database using a BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>). The isolates with an identity >99% for a type strain were considered to be the same species. The reference strains and their accession numbers for *rpoB* gene sequencing included *A. aquariorum* CECT 7289<sup>T</sup>/FJ481643.1, *A. hydrophila* ATCC 7966<sup>T</sup>/AY851091.1, *A. veronii* biovar *veronii* ATCC 35624<sup>T</sup>/AY851122.1, *A. punctata* V83/AY851107.1, *A. taiwanensis* A2-50<sup>T</sup>/JF 972599.1, and *A. sanarellii* A2-067<sup>T</sup>/JF972600.1. For this study, all these isolates were re-identified by using both commercially automated identification systems: Vitek 2 GN cards and Phoenix system (NMIC/ID-72 cards) (Becton-Dickinson Microbiology Systems) along with the MALDI Biotyper system. Furthermore, two additional biochemical tests, L-arabinose and Voges-Proskauer (VP) reactions were also performed to phenotypically differentiate three subspecies of *A. hydrophila*, i.e. *A. hydrophila* subsp. *dhakensis* (*A. dhakensis*), *A. hydrophila* subsp. *hydrophila* and *A. hydrophila* subsp. *ranae* (2). Isolates with positive reactions of both VP and L-arabinose were considered as *A. hydrophila* subsp. *hydrophila*. Isolates with negative reaction for both reactions were identified as *A. hydrophila* subsp. *ranae* (2). Isolates with positive reaction of VP but negative for L-arabinose were *A. dhakensis*.

#### SUPPLEMENTAL REFERENCES

1. Kupfer M, Kuhnert P, Korczak BM, Peduzzi R, Demarta A. 2006. Genetic relationships of *Aeromonas* strains inferred from 16S rRNA, *gyrB* and *rpoB* gene

sequences. *Int J Syst Evol Microbiol* **56**: 2743-2751.

2. **Beaz-Hidalgo R, Martinez-Murcia A, Figueras MJ.** 2013. Reclassification of *Aeromonas hydrophila* subsp. *dhakensis* Huys et al. 2002 and *Aeromonas aquariorum* Martinez-Murcia et al. 2008 as *Aeromonas dhakensis* sp. nov. comb nov. and emendation of the species *Aeromonas hydrophila*. *Syst Appl Microbiol* **36**: 171-176.

**SUPPLEMENTARY TABLE 1.** *In vitro* susceptibilities of the 217 clinical isolates of *Aeromonas* species to antimicrobial agents by Phoenix system (NMIC/ID-72 Combo cards).

species/agents	<i>A. dhakensis</i> (n=58)					<i>A. hydrophila</i> (n=35)					<i>A. veronii</i> (n=61)				<i>A. caviae</i> (n=61)					
	MIC (µg/ml)		% of isolates			MIC (µg/ml)		% of isolates			MIC (µg/ml)		% of isolates		MIC (µg/ml)		% of isolates			
	range	50%	90%	S	R	range	50%	90%	S	R	range	50%	90%	S	R	range	50%	90%	S	R
Ampicillin/sulbactam	>16/8	>16/8	>16/8	0	100	>16/8	>16/8	>16/8	0	100	4/2->16/8	>16/8	>16/8	3.3	96.7	8/4->16/8	>16/8	>16/8	1.6	98.4
Cefazolin	4->16	>16	>16			4->16	>16	>16			4->16	≤4	4			8->16	>16	>16		
Cefotaxime	2->32	≤2	4			2->16	≤2	>16			2	≤2	2			2->16	≤2	>16		
Ceftriaxone	4->32	≤4	16			4->32	≤4	32			4->32	≤4	4			4->32	≤4	>32		
Ceftazidime	0.5->16	≤0.5	1	93.1	5.2	0.5->16	≤0.5	4	91.4	8.6	0.5	≤0.5	0.5	100	0	0.5->16	1	>16	72.1	19.7
Cefepime	2	≤2	2	100	0	2->16	≤2	2	94.3	5.7	2	≤2	2	100	0	2->16	≤2	>16	83.6	14.8
Piperacillin/tazobactam	4/4->64/4	≤4/4	4/4	89.7	8.6	≤4/4	≤4/4	≤4/4	100	0	4/4->64/4	≤4/4	4/4	98.4	1.6	4/4->64/4	≤4/4	64/4	80.3	3.3
Ertapenem	0.5->4	≤0.5	>4	84.5	12.1	0.5-2	≤0.5	0.5	100	0	0.5	≤0.5	0.5	100	0	0.5->4	≤0.5	0.5	91.8	6.6
Imipenem	1->8	≤1	1	98.3	1.7	1	≤1	1	100	0	1	≤1	1	100	0	1-4	≤1	1	98.4	1.6
Meropenem	1->8	≤1	1	98.3	1.7	1	≤1	1	100	0	1	≤1	1	100	0	1	≤1	1	100	0
Aztreonam	2-4	≤2	2	100	0	2->16	≤2	2	94.3	5.7	2	≤2	2	100	0	2->16	≤2	4	91.8	8.2
Gentamicin	2->8	4	8	74.1	6.9	2->8	≤2	>8	88.6	11.4	2-8	≤2	4	96.7	0	2->8	≤2	>8	82.0	13.1
Amikacin	8-32	16	16	91.4	0	8->32	≤8	8	94.3	5.7	8-16	≤8	8	100	0	8-32	≤8	8	98.4	0
Levofloxacin	1-2	≤1	1	100	0	1->4	≤1	1	97.1	2.9	1	≤1	1	100	0	1->4	≤1	4	86.9	8.2
Ciprofloxacin	0.5->2	≤0.5	1	91.4	5.2	0.5->2	≤0.5	2	85.7	8.6	0.5	≤0.5	0.5	100	0	0.5->2	≤0.5	>2	78.7	16.4
Trimethoprim/sulfamethoxazole	0.5/9.5->2/38	≤0.5/9.5	>2/38	81.0	19.0	0.5/9.5-1/19	≤0.5/9.5	1/19	100	0	0.5/9.5->2/38	≤0.5/9.5	0.5/9.5	91.8	8.2	0.5/9.5->2/38	≤0.5/9.5	>2/38	75.4	24.6

S, susceptible; R, resistant.

**SUPPLEMENTARY FIGURE 1.** Spectra generated by MALDI Biotyper system for *A. dhakensis*, *A. caviae*, *A. veronii*, *A. hydrophila* subsp. *hydrophila*, *A. sanarellii*, and *A. taiwanesis*.

