

***Acinetobacter thutiue* sp. nov. isolated from oil-contaminated soil in motorbike repair workshops**

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Running title: *Acinetobacter thutiue* sp. nov.

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Figure S1. Sampling sites at the automobile workshops.

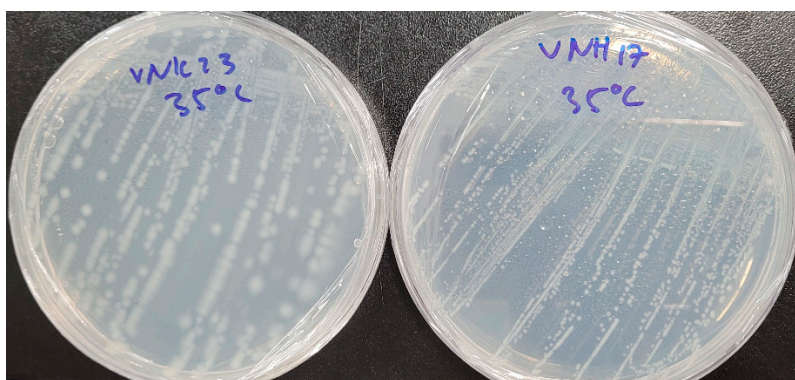


Figure S2. Colonies of *A. thutiue* VNH17^T and *A. thutiue* VNK23 were grown on R2A at 35°C for 48 h.

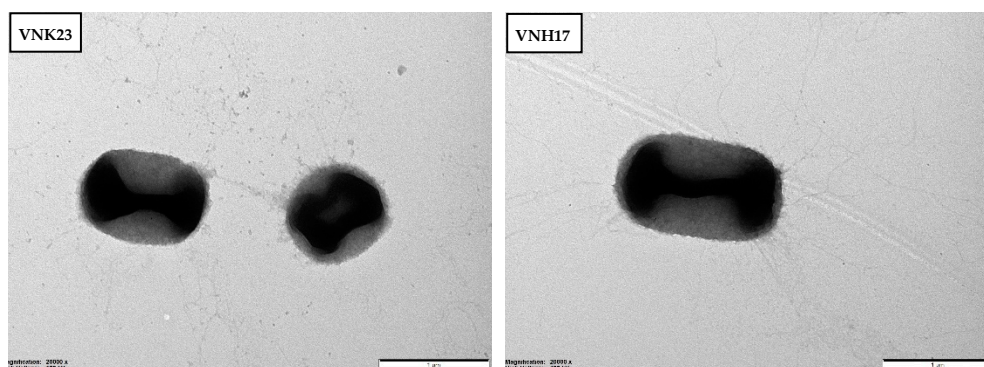


Figure S3. Transmission electron microscopy of the *A. thutiue* VNH17^T and *A. thutiue* VNK23 strains grown on R2A medium plates for 2 days at 30°C and Bar 1 μm.

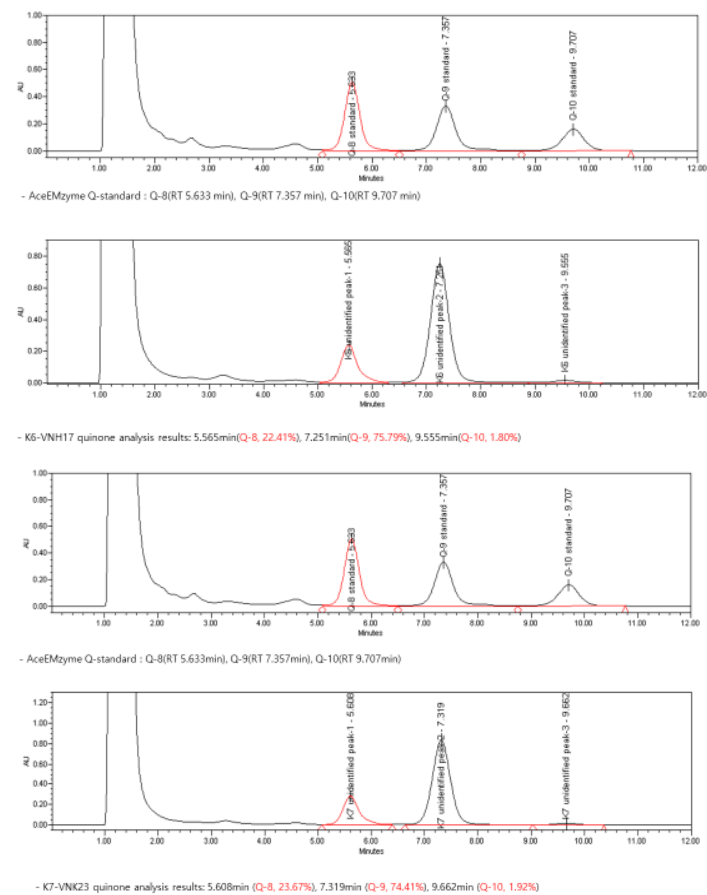


Figure S4. Quinone analysis results of the *A. thutiae* VNH17^T and *A. thutiae* VNK23 strains.

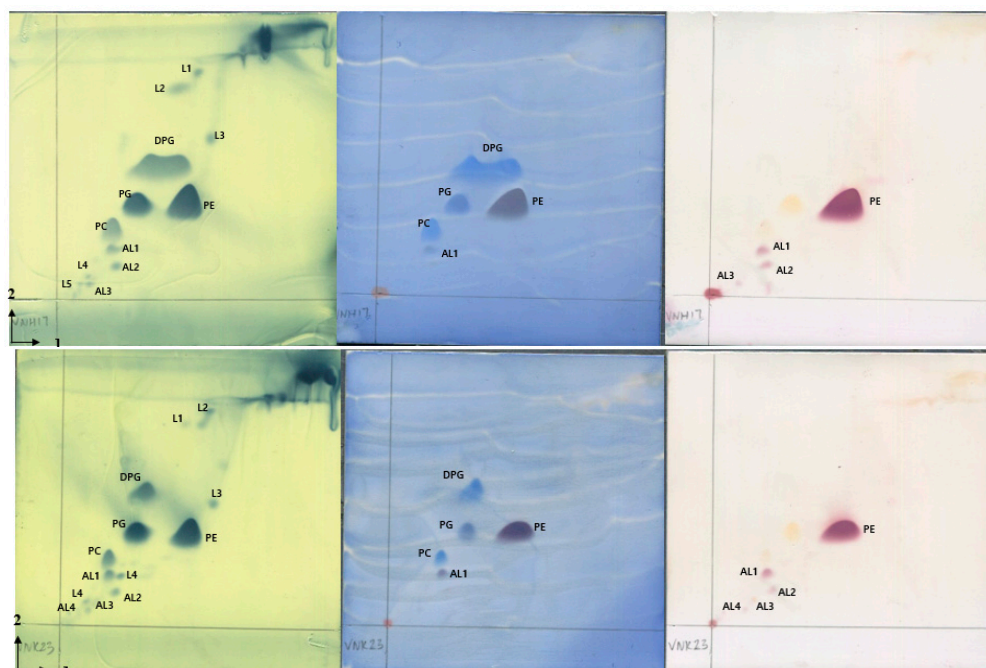


Figure S5. Polar lipid profile of the *A. thutiae* VNH17^T and *A. thutiae* VNK23 strains
PG - phosphatidylglycerol; DPG - diphosphatidylglycerol; PE - phosphatidylethanolamine; PC – phodphatidylcholine, AL1-4 – unidentified aminolipid, L1-5 – unidentified polar lipids

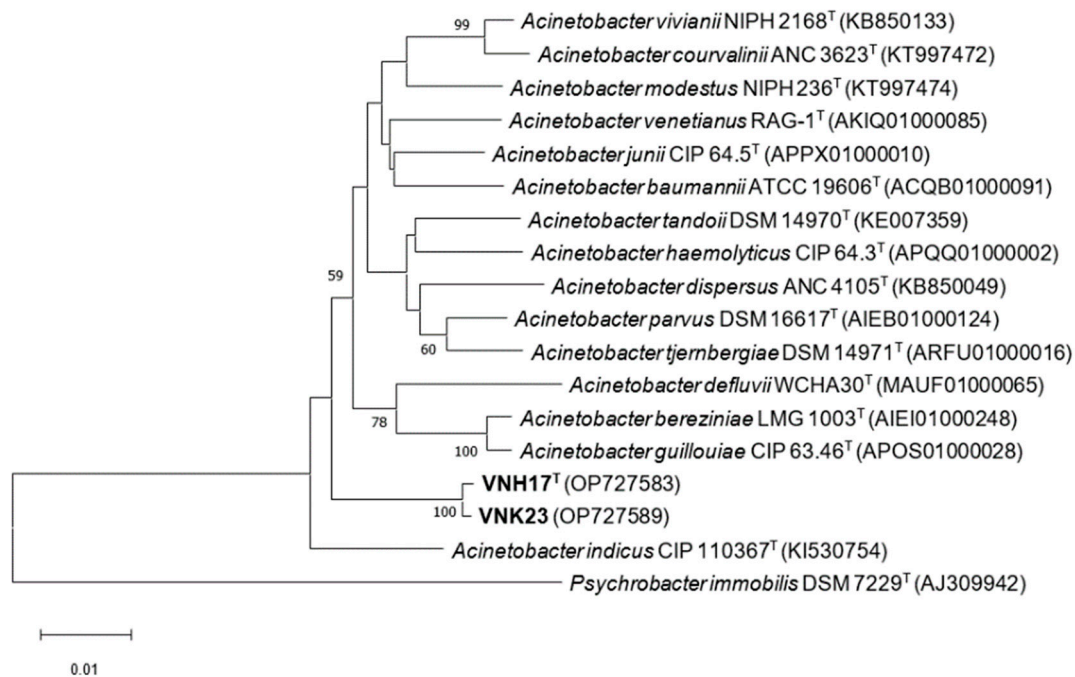


Figure S6. The phylogenetic tree was reconstructed with the neighbor-joining method based on 16S rRNA gene sequences of VNH17^T, VNK23, and the type strains of the genus *Acinetobacter*. *Moraxella lacunata* NCTC 7911^T was used as an outgroup. Numbers at branch nodes represent confidence levels (values > 50% are shown) from 1000 replicate bootstrap samplings. GenBank accession numbers are shown in parentheses. Bar, 0.01 substitutions per nucleotide position.

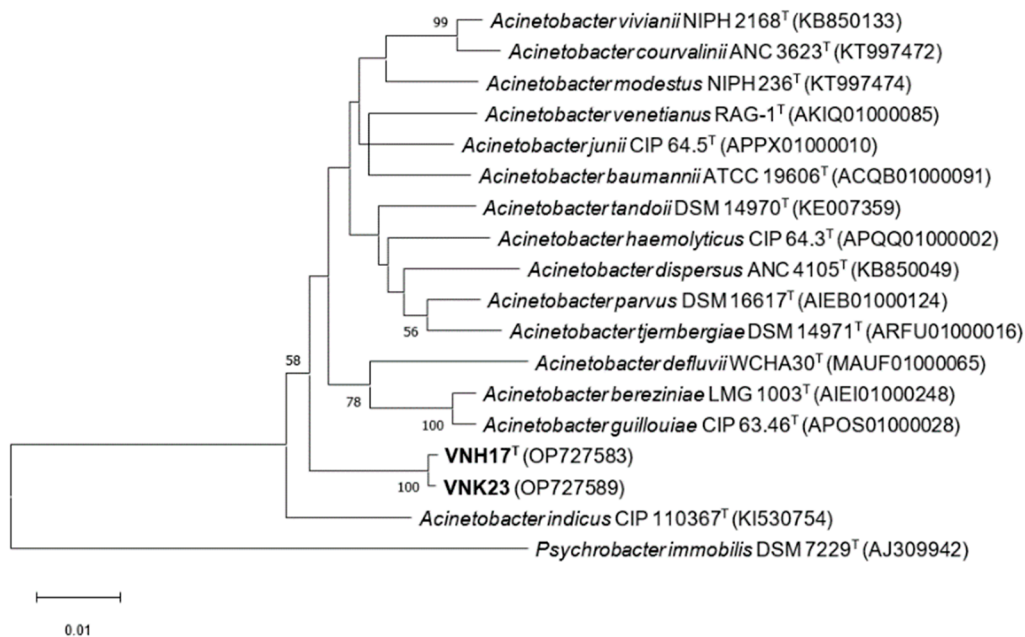


Figure S7. Phylogenetic tree reconstructed with the minimum evolution method based on 16S rRNA gene sequences of the two isolates and type strains of the genus *Acinetobacter*. *Psychrobacter immobilis* DSM 7229^T was used as an outgroup. Numbers at branch nodes represent confidence levels (values > 50% are shown) from 1000 replicate bootstrap samplings. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA 11.

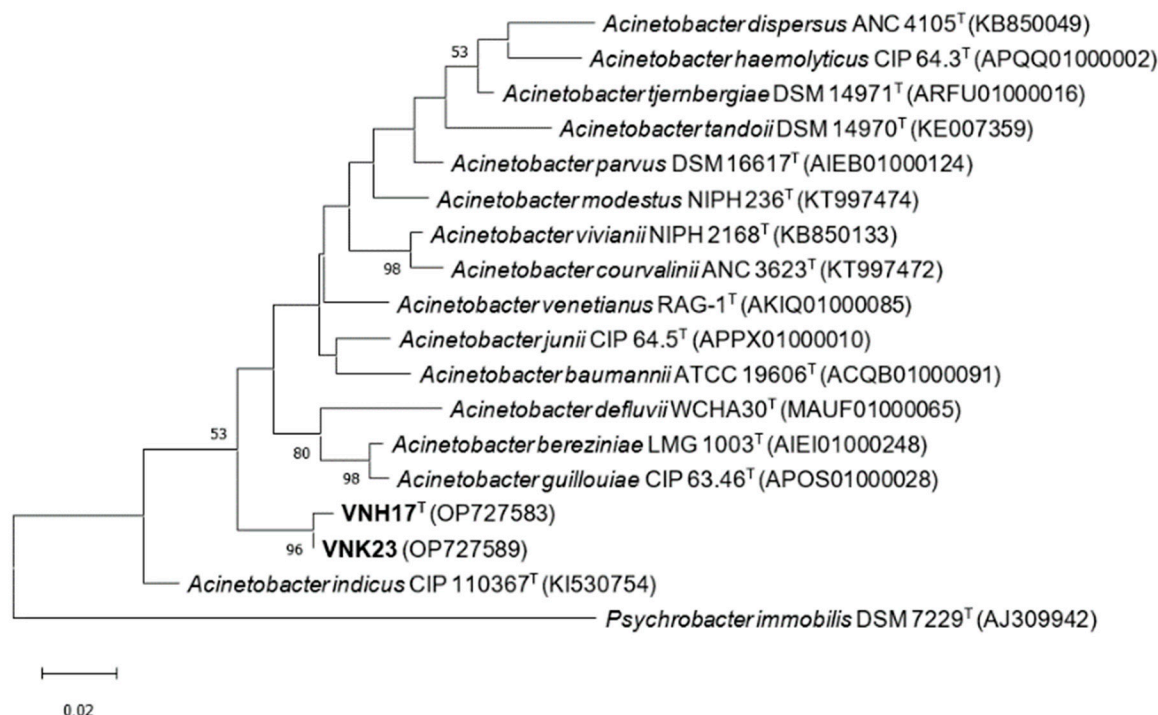


Figure S8. Phylogenetic tree reconstructed with the maximum likelihood method based on 16S rRNA gene sequences of the two isolates and type strains of the genus *Acinetobacter*. *Psychrobacter immobilis* DSM 7229^T was used as an outgroup. Numbers at branch nodes represent confidence levels (values >50% are shown) from 1000 replicate bootstrap samplings. GenBank accession numbers are given in parentheses. Bar, 0.02 substitution per nucleotide position. Evolutionary analyses were conducted in MEGA 11.

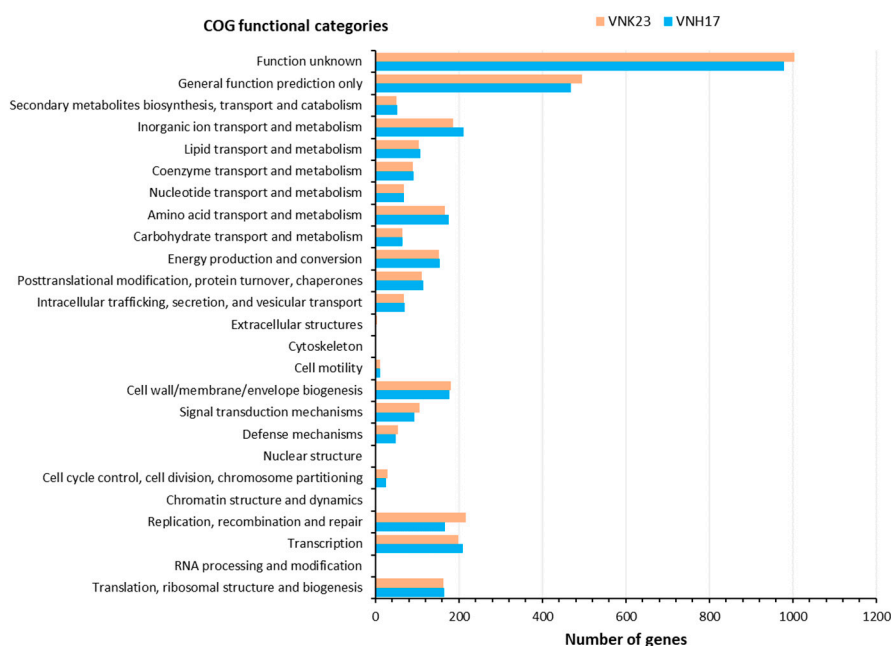


Figure S9. COG functional classification of proteins in the *A. thutiae* VNH17^T and *A. thutiae* VNK23 genome.

Table S1. Zone diameter breakpoint (mm) of antimicrobial agents for *Acinetobacter thutiae* VNH17^T and VNK23.

Strain	Piperacillin	Ampicillin – sulbactam	Gentamicin	Tetracycline	Levofloxacin
VNH17 ^T	13-15 (R)	≥ 23 (S)	13-14 (I)	25-27 (S)	≥ 28 (S)
VNK23	≥ 21 (S)	35 (S)	≥ 17 (S)	≥ 31 (S)	≥ 28 (S)

Susceptibility categories (S, susceptible; I, intermediate; R, resistant) according to the Clinical and Laboratory Standards Institute (CLSI) are shown.

Table S2. Results from the API ZYM, API 20NE test. (1) VNH17^T; (2) VNK23; (3) *Acinetobacter parvus* KACC 12455; (4) *Acinetobacter junii* KACC12228; (5) *Acinetobacter tandoii* KACC 12229; (6) *Acinetobacter indicus* KCTC 42000; (7) *Acinetobacter bereziniae* KCTC 42001; (8) *Acinetobacter venetianus* DSM23050. Data are from the present study. +, positive; -, negative, +/-, weak positive or ambiguous.

Characteristics	1	2	3	4	5	6	7	8
Enzymatic reaction (API ZYM)								
Alkaline phosphate	-	-	-	+	+	+	+	+
Esterase	+	+	+	+	+/-	+	+	+
Esterase lipase (C8)	+	+	+	+	+	+	+	+
Lipase (C14)	+	+	-	-	+	+/-	+	+
Leucine arylamidase	+	+	+	+	+	+	+	+
Valine arylamidase	+	+	+/-	+	+	+	+	+
Crystine arylamidase	+/-	+/-	-	-	+	+	+	+
Trypsin	-	-	-	-	-	-	-	-
Alpha chymotrypsin	-	-	-	-	-	-	-	-
Acid phosphatase	+	+	+	+	+	+/-	+	+/-
Naphthol-AS-BI- phosphate	+	+	+	+	+	+	+	+
Alpha galactosidase	-	-	-	-	-	-	-	-
Beta galactosidase	-	-	-	-	-	-	-	-
Beta glucuronidase	-	-	-	-	-	-	-	-
Alpha glucosidase	-	-	-	-	-	-	+/-	-
Beta glucosidase	-	-	-	-	-	-	-	-
N-acetyl beta glucosaminidase	-	-	-	-	-	-	-	-
Alpha monosidase	-	-	-	-	-	-	-	-
Alpha fucosidase	-	-	-	-	-	-	-	-
API 20NE								
Nitrate reduction	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-
Glucose fermentation	-	-	-	-	-	-	+/-	-
Arginine dihydrolase	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+
Aesculin hydrolysis	-	-	-	-	-	+	+	-
Gelatin hydrolysis	-	-	-	-	-	-	-	+/-
β - Galactosidase	-	-	-	-	-	+	-	-
Assimilation of								
D-Glucose	-	-	-	-	-	-	-	-
L-Arabinose	-	-	-	-	-	-	-	-
D-Mannose	-	-	-	-	-	+	-	-
D-Mannitol	-	-	-	-	-	-	+	-

Characteristics	1	2	3	4	5	6	7	8
N-Acetyl-glucosamine	-	-	-	-	-	+	-	-
D-Maltose	-	-	-	-	-	+	+	-
Potassium gluconate	-	-	-	-	-	-	+	-
Capric acid	+	+	+	+	+	+/-	+	+
Adipic acid	-	-	-	-	-	-	+	-
Malate	-	-	-	+	+	+	+	+
Trisodium citrate	+	+	-	+	-	-	+	+
Phenylacetic acid	-	-	-	-	+	+	-	-
API 32								
L-Rhamnose	-	-	-	-	-	-	-	-
N-Acetyl-glucosamine	-	-	-	-	-	-	-	-
D-Ribose	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-
D-Saccharose (sucrose)	-	-	-	-	-	-	-	-
D-Maltose	-	-	-	-	-	-	-	-
Itaconic acid	-	-	-	-	-	-	-	-
Suberic acid	-	-	-	-	-	-	+	-
Sodium malonate	-	-	-	-	+	-	-	+
Sodium acetate	+	+	+	+	+	+	+	+
Lactic acid	-	-	-	-	+	+	+	-
L-Alanine	+/-	-	-	-	+	+	+	+
Potassium 5-ketogluconate	-	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-
3-Hydroxybenzoic acid	-	-	-	-	-	-	-	-
L-Serine	-	-	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-	+/-	-
D-Glucose	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	+	-
L-Arabinose	-	-	-	-	-	-	-	-
Propionic acid	+	+	-	+	+	+	+	+
Capric acid	+	+	+	+	+	+	+	+
Valeric acid	+	+	+	+	+	+	+	+
Trisodium citrate	+	+	-	+	-	-	+	+
L-Histidine	-	-	-	+	+	-	+	+
Potassium 2-ketogluconate	-	-	-	-	-	-	-	-
3-Hydroxybutyric acid	-	-	-	-	+	+/-	+	+
4-Hydroxybenzoic acid	-	-	-	-	+	-	+	-
L-Proline	+	+	-	+	+	-	+	+
Source of isolation	oil-contaminated soil	oil-contaminated soil	human clinical specimens	human clinical specimens	activated sludge	hexachlorocyclohexane dump site	human wound	seawater
DNA G+C content (mol%)	42.1	41.8	41.8	42	40	40.4	38.2	43.9

Table S3. ANIb and dDDH values (%) between the genome sequences of the two isolates and other *Acinetobacter* species with validly published names.

No.	Taxon name	Strain name	Genome accession no.	ANIb	dDDH	ANIb	dDDH
				VNH17 ^T		VNK23	
1	<i>A. albensis</i>	ANC 4874 ^T	FMBK00000000.1	75.05	20.30	74.12	20.30
2	<i>A. amyesii</i>	ANC 5579 ^T	JAMCOU010000039.1	74.54	22.20	74.39	22.00
3	<i>A. apis</i>	ANC 5114 ^T	FZLN00000000.1	72.19	20.20	72.32	20.40
4	<i>A. barettiae</i>	B10A ^T	VTDM00000000.1	75.76	21.10	71.75	21.00
5	<i>A. baumannii</i>	ATCC 19606 ^T	CP046654.1	75.76	20.60	75.86	20.70
6	<i>A. baylyi</i>	CIP 107474 ^T	APPT00000000.1	74.80	20.80	74.81	20.90
7	<i>A. beijerinckii</i>	CIP 110307 ^T	APQL00000000.1	80.30	22.90	80.27	23.20
8	<i>A. bereziniae</i>	CIP 70.12 ^T	APQG00000000.1	74.24	21.50	74.43	21.90
9	<i>A. bohemicus</i>	ANC 3994 ^T	APOH00000000.1	74.46	20.70	74.64	20.30
10	<i>A. boissieri</i>	ANC 4422 ^T	FMYL00000000.1	72.02	20.70	71.85	21.50
11	<i>A. bouvetii</i>	JCM 18991 ^T	AP024595.1	73.82	20.30	73.61	20.00
12	<i>A. brisouii</i>	ANC 4119 ^T	APPR00000000.1	74.24	21.40	74.10	21.70
13	<i>A. calcoaceticus</i>	CIP 81.8 ^T	APQI00000000.1	75.83	20.70	75.70	20.80
14	<i>A. celticus</i>	ANC 4603 ^T	MBDL00000000.1	73.92	20.20	73.96	20.20
15	<i>A. chengduensis</i>	WCHAc060005 ^T	RCHC00000000.1	73.95	20.70	73.92	20.30
16	<i>A. chinensis</i>	WCHAc010005 ^T	CP032134.1	74.31	20.90	74.44	21.60
17	<i>A. colistiniresistens</i>	NIPH 2036 ^T	ATGK00000000.1	81.26	24.30	81.47	24.60
18	<i>A. courvalinii</i>	ANC 3623 ^T	APSA00000000.1	81.40	24.10	81.37	24.00
19	<i>A. cumulans</i>	WCHAc060092 ^T	CP035934.2	73.75	20.90	74.06	21.10
20	<i>A. defluvii</i>	WCHA30 ^T	CP029397.2	74.44	21.60	74.59	21.70
21	<i>A. dispersus</i>	ANC 4105 ^T	APRL00000000.1	81.24	24.10	81.30	24.30
22	<i>A. equi</i>	114 ^T	CP012808.1	74.37	20.40	74.41	20.40
23	<i>A. gandensis</i>	ANC 4275 ^T	LZDS00000000.1	74.16	20.80	76.14	20.50
24	<i>A. geminorum</i>	J00019 ^T	JABELE00000000.1	75.95	20.80	76.14	21.20
25	<i>A. gernerii</i>	CIP 107464 ^T	APPN00000000.1	74.05	21.70	74.41	22.10
26	<i>A. guerrae</i>	AC1271 ^T	LXGN00000000.1	75.86	21.60	75.48	21.20
27	<i>A. guillouiae</i>	CIP 63.46 ^T	APOS00000000.1	74.72	21.70	74.28	21.30
28	<i>A. gyllenbergii</i>	CIP 110306 ^T	ATGG00000000.1	81.18	24.00	81.37	24.30
29	<i>A. haemolyticus</i>	CIP 64.3 ^T	APQQ00000000.1	79.61	22.70	79.61	22.60
30	<i>A. halotolerans</i>	JCM 31009 ^T	SGIM00000000.1	79.95	22.80	80.10	22.80
31	<i>A. harbinensis</i>	HITLi7 ^T	JXBK00000000.1	74.10	21.00	74.25	20.70
32	<i>A. ihumii</i>	Marseille-P8049 ^T	UYYC00000000.1	75.46	21.00	75.56	20.90
33	<i>A. indicus</i>	ANC 4215 ^T	ATGH00000000.1	75.10	22.00	75.31	22.20
34	<i>A. johnsonii</i>	CIP 64.6 ^T	APON00000000.1	74.05	21.10	74.08	20.90
35	<i>A. junii</i>	CIP 64.5 ^T	APPX00000000.1	79.53	22.40	79.56	22.50
36	<i>A. kanungonis</i>	PS-1 ^T	JAACKC00000000.1	75.20	21.30	74.97	21.20
37	<i>A. kookii</i>	ANC 4667 ^T	FMYO00000000.1	74.70	20.30	74.91	20.30
38	<i>A. lactucae</i>	NRRLB-41902 ^T	LRPE00000000.1	75.81	20.70	75.87	20.80
39	<i>A. lanii</i>	185 ^T	CP049916.1	73.85	21.40	73.82	21.20
40	<i>A. larvae</i>	BRTC-1 ^T	CP016895.1	72.79	21.60	72.65	21.10
41	<i>A. lwoffii</i>	NIPH 512 ^T	AYHO00000000.1	74.98	22.70	74.49	22.00
42	<i>A. modestus</i>	CCM 8639 ^T	BMDV00000000.1	81.30	23.90	81.29	24.00

43	<i>A. nectaris</i>	CIP 110549 ^T	AYER00000000.1	72.56	21.20	72.50	21.50
44	<i>A. nematophilus</i>	A-IN1 ^T	JAPKMY00000000.1	73.99	20.50	74.21	20.80
45	<i>A. nosocomialis</i>	NIPH 2119 ^T	APOP00000000.1	75.71	20.70	75.59	20.40
46	<i>A. parvus</i>	CIP 108168 ^T	APOM00000000.1	91.70	45.40	91.95	45.20
47	<i>A. piscicola</i>	LW15 ^T	NIFO00000000.1	74.62	21.50	74.62	21.40
48	<i>A. pittii</i>	CIP 70.29 ^T	APQP00000000.1	75.71	20.60	75.73	20.50
49	<i>A. pollinis</i>	SCC477 ^T	VTDQ00000000.1	72.71	20.40	72.57	20.40
50	<i>A. populi</i>	PBJ7 ^T	NEXX00000000.1	73.00	21.50	72.76	21.40
51	<i>A. portensis</i>	AC877 ^T	LWRV00000000.1	74.54	20.90	74.56	21.60
52	<i>A. pragensis</i>	ANC 4149 ^T	LUAW00000000.1	73.58	20.00	73.47	19.90
53	<i>A. proteolyticus</i>	NIPH 809 ^T	APOI00000000.1	81.33	24.30	81.21	24.20
54	<i>A. pseudolwoffii</i>	ANC 5044 ^T	PHRG00000000.1	74.39	21.00	74.67	21.40
55	<i>A. puyangensis</i>	ANC 4466 ^T	OANT00000000.1	72.90	21.20	72.88	21.00
56	<i>A. qingfengensis</i>	CCUG 69710 ^T	VXKN00000000.1	72.81	22.00	72.76	22.30
57	<i>A. radioresistens</i>	CIP 103788 ^T	APQF00000000.1	74.05	20.70	74.20	20.40
58	<i>A. rathckeae</i>	EC24 ^T	VTDO00000000.1	72.14	20.60	72.18	20.60
59	<i>A. rudis</i>	CIP 110305 ^T	ATGI00000000.1	72.98	21.40	73.14	21.20
60	<i>A. schindleri</i>	CIP 107287 ^T	APPQ00000000.1	74.80	22.50	74.67	22.10
61	<i>A. sedimenti</i>	A3.8 ^T	JAKUML00000000.1	73.05	23.20	73.07	24.10
62	<i>A. seifertii</i>	NIPH 973 ^T	APOO00000000.1	75.80	20.60	75.88	20.40
63	<i>A. shaoyimingii</i>	323-1 ^T	CP049801.1	73.87	21.30	74.01	21.40
64	<i>A. sichuanensis</i>	WCHAc060041 ^T	PYIX00000000.2	74.26	21.20	74.37	21.30
65	<i>A. silvestris</i>	ANC 4999 ^T	NEGB00000000.1	74.34	20.80	74.36	20.60
66	<i>A. soli</i>	CIP 110264 ^T	APPU00000000.1	74.25	20.50	74.66	20.60
67	<i>A. stercoris</i>	KPC-SM-21 ^T	OOGT00000000.1	74.02	21.10	74.24	20.90
68	<i>A. tandoii</i>	CIP 107469 ^T	AQFM00000000.1	74.55	20.30	74.49	20.40
69	<i>A. terrae</i>	ANC 4282 ^T	JABERI00000000.1	74.86	21.10	74.95	20.80
70	<i>A. terrestris</i>	ANC4471 ^T	SJNZ00000000.1	74.68	20.40	74.64	20.20
71	<i>A. tibetensis</i>	Y-23 ^T	ASM2382431v1	74.39	20.60	74.41	20.70
72	<i>A. tjernbergiae</i>	DSM 14971 ^T	ARFU00000000.1	81.29	23.90	81.24	24.00
73	<i>A. towneri</i>	CIP 107472 ^T	APPY00000000.1	74.83	21.50	75.05	21.60
74	<i>A. ursingii</i>	CIP 107286 ^T	APQA00000000.1	75.67	22.20	75.72	21.30
75	<i>A. variabilis</i>	NIPH 2171 ^T	APRS00000000.1	74.85	21.60	74.55	21.50
76	<i>A. venetianus</i>	CIP 110063 ^T	APPO00000000.1	80.52	23.10	80.42	23.20
77	<i>A. vivianii</i>	NIPH 2168 ^T	APRW00000000.1	81.52	24.40	81.51	24.30
78	<i>A. wanghuiae</i>	dk386 ^T	CP045650.1	74.08	20.80	73.87	20.50
79	<i>A. wuhouensis</i>	WCHA60 ^T	CP031716.1	74.47	21.60	74.41	21.70