NOTE

***Aeromonas hydrophila* subsp. *dhakensis* subsp. nov., isolated from children with diarrhoea in Bangladesh, and extended description of *Aeromonas hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980)**

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A group of 10 *Aeromonas hydrophila*-like strains referred to as group BD-2, which mainly comprised haemolytic and cytotoxic isolates from diarrhoeal children in Bangladesh, was subjected to a polyphasic taxonomic study including fluorescent amplified fragment length polymorphism (FAFLP) and ERIC-PCR fingerprinting, microplate DNA–DNA hybridization and extensive phenotypic characterization. Comparison of FAFLP and ERIC-PCR genotypic profiles indicated that the group BD-2 isolates constituted a separate taxon that was most closely related to *A. hydrophila* DNA hybridization group (HG) 1. Determination of DNA–DNA hybridization values with type and reference strains of all known *Aeromonas* taxa revealed that the BD-2 group represented a homogeneous taxon (internal DNA homology, 77–94%) that exhibited 78–92% DNA relatedness with the type strain of *A. hydrophila* HG1. These results indicated that the BD-2 group belongs genotypically to *A. hydrophila* HG1, but examination of 152 phenotypic characteristics revealed that the group BD-2 isolates differed from representatives of HG1 in eight biochemical properties. The diagnostic value of at least three of these tests, namely utilization of urocanic acid and L-arabinose and acid production from L-arabinose, has been confirmed outside this study by other workers. Collectively, the genotypic and phenotypic data reported suggest that the BD-2 group represents a subspecies of *A. hydrophila*, for which the name *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov. is proposed. Its type strain is P21^T (= LMG 19562^T = CCUG 45377^T). An extended description of *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980) is given.

Keywords: *Aeromonas hydrophila*, DNA–DNA hybridization, FAFLP, diarrhoea

Members of the genus *Aeromonas* are ubiquitous in most types of fresh water including lake and river water, domestic sewage and raw and processed drinking water (Holmes *et al.*, 1996). Some mesophilic *Aeromonas* species have been suspected of being

opportunistic human pathogens, especially in association with gastrointestinal diseases including traveller's diarrhoea and paediatric gastroenteritis (Joseph, 1996). Although there is an overwhelming amount of clinical and molecular information pointing to the production of a wide range of structural and extracellular virulence factors by clinical *Aeromonas* isolates (Janda, 1991), the virulence mechanism leading to human bacteraemia or diarrhoeal disease has not been

Abbreviation: FAFLP, fluorescent amplified fragment length polymorphism.

Table 1. *Aeromonas* type-BD-2 strains included in this study

Strain	Original no.*	Origin	Virulence factors†	
			Haemolysin	Cytotoxin titre
LMG 19558	P11	Faeces of diarrhoeal child	+	1/8
LMG 19559	P71	Faeces of diarrhoeal child	+	1/512
LMG 19560	P17C	Faeces of control child	+	1/512
LMG 19561	P76	Faeces of diarrhoeal child	—	0
LMG 19562 ^T	P21 ^T	Faeces of diarrhoeal child	+	1/512
LMG 19563	P31	Faeces of diarrhoeal child	+	1/512
LMG 19564	P65	Faeces of diarrhoeal child	+	1/256
LMG 19565	P56	Faeces of diarrhoeal child	+	1/1024
LMG 19566	P50	Faeces of diarrhoeal child	+	1/1024
LMG 19567	P48	Faeces of diarrhoeal child	+	1/1024

* Strain designation used by Kühn *et al.* (1997).

† Haemolytic and cytotoxic effects respectively determined on sheep erythrocytes and Vero cells (Kühn *et al.*, 1997).

Table 2. DNA relatedness between the *Aeromonas* BD-2 group and type and reference strains of other *Aeromonas* taxa

Results are percentages and are expressed as means of four determinations. Reciprocal hybridizations showed a maximum standard deviation of 6%, whereas repeated experiments exhibited a maximum standard deviation of 3%.

Strain	Source of labelled DNA						
	1	2	3	4	5	6	7
1. <i>Aeromonas</i> BD-2 LMG 19559	100	94	90	94			
2. <i>Aeromonas</i> BD-2 LMG 19562 ^T	81	100	88	79	82	79	85
3. <i>Aeromonas</i> BD-2 LMG 19564	77	84	100	82	85	81	89
4. <i>Aeromonas</i> BD-2 LMG 19565	84	89	94	100	92	90	94
5. <i>A. hydrophila</i> HG1 LMG 2844 ^T		78	81	84	100		
6. <i>A. hydrophila</i> HG1 LMG 13443		77	83	85		100	
7. <i>A. hydrophila</i> HG1 LMG 13658		77	78	82			100
8. <i>Aeromonas</i> spp.*		33–65	31–65	33–67			

* Represented by *Aeromonas bestiarum* LMG 13444^T, *Aeromonas salmonicida* subsp. *salmonicida* LMG 3780^T, *Aeromonas caviae* LMG 3775^T, *Aeromonas media* LMG 9073^T, *Aeromonas eucrenophila* LMG 3774^T, *Aeromonas sobria* LMG 3783^T, *Aeromonas jandaei* LMG 12221^T, *Aeromonas veronii* LMG 9075^T, *Aeromonas encheleia* LMG 16330^T, *Aeromonas schubertii* LMG 9074^T, *Aeromonas trota* LMG 12223^T and *Aeromonas popoffii* LMG 17541^T.

clearly established. In this regard, the initiation of retrospective studies is a promising attempt to learn about the pathogenic potential and spread of infectious *Aeromonas* strains (Reina *et al.*, 1993; Barillo *et al.*, 1996), although the success of this approach may be hampered to some extent by the lack of reliable taxonomic data in earlier case reports.

In a previous study initiated by the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh (Kühn *et al.*, 1997), a set of *Aeromonas* isolates originating from humans with diarrhoea, healthy controls and surface water were compared

with regard to their virulence properties and their taxonomic identity. Biochemical fingerprinting data obtained with the PhenePlate (PhP) system demonstrated that one PhP type, named type BD-2, was strongly dominant among patient isolates but was found very rarely in control samples or in the environment. Strains of type BD-2 displayed high haemolytic and cytotoxic activities and were identified by fatty acid analysis as members of *Aeromonas hydrophila* DNA hybridization group (HG) 1. Comparison with a genotypic database generated from amplified fragment length polymorphism (AFLP) fingerprinting patterns also allocated the BD-2 isolates to *Aeromonas*

Fig. 1. Dendrogram showing the relative genotypic position of the 10 group BD-2 isolates in the genus *Aeromonas* obtained after comparison with the laboratory-based AEROLIB database comprising 102 type and reference strains of all currently accepted *Aeromonas* species (Huys & Swings, 1999). Clustering was obtained following numerical analysis of digitized FAFLP band patterns using Pearson's product-moment correlation coefficient (expressed as percentages) and the unweighted paired group method using arithmetic means.

HG1, although clustering analysis of digitized AFLP patterns indicated that BD-2 strains constituted a subcluster adjacent to the taxonomic core of *A. hydrophila* HG1, including the type strain (G. Huys, unpublished data).

The current investigation was undertaken to assess accurately the affiliation of the type BD-2 strains with *A. hydrophila* HG1 using a polyphasic taxonomic approach. For this purpose, a selection of 10 BD-2 strains was subjected to fluorescent AFLP (FAFLP) and ERIC-PCR fingerprinting, microscale DNA-DNA hybridization and extensive phenotypic charac-

terization. The data generated indicate that the BD-2 group constitutes a subspecies of *A. hydrophila*, for which the name *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov. is proposed. In addition, an extended description of *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980) is given.

Ten of 25 strains that were described previously as *A. hydrophila* HG1 type BD-2 (Kühn *et al.*, 1997) were included in the present study and are listed in Table 1. Type and reference strains of all unique *Aeromonas* species (Table 2; Figs 1 and 2) were obtained from the

Fig. 2. Digital photograph showing ERIC-PCR fingerprints of *A. hydrophila* HG1 strains, type BD-2 isolates and reference strains of *Aeromonas bestiarum* and *A. hydrophila* HG3. Lanes: 1 and 19, SmartLadder band sizing mixture (Eurogentec); 2–6, *A. hydrophila* HG1 strains LMG 2844^T, LMG 13443, LMG 13656, LMG 13658 and LMG 13660; 7–16, BD-2 group isolates LMG 19558, LMG 19559, LMG 19560, LMG 19561, LMG 19562^T, LMG 19563, LMG 19564, LMG 19565, LMG 19566 and LMG 19567; 17, *A. bestiarum* LMG 13444^T; 18, *A. hydrophila* HG3 LMG 13451. Arrows indicate the two ERIC-PCR markers that allow differentiation of HG1 strains from group BD-2 isolates upon repeated ERIC-PCR assays.

BCCM/LMG Bacteria Collection, Ghent University, Belgium. All strains were cultured aerobically on Trypticase soy agar (TSA), containing 3% (w/v) Trypticase soy broth (BBL) and 1.5% (w/v) bacteriological agar no. 1 (Oxoid), at 28 °C for 24 h. All BD-2 isolates were subjected to FAFLP fingerprinting and the resulting FAFLP profiles were compared with the laboratory-based AEROLIB database (Huys & Swings, 1999). Microscale DNA extraction, AFLP template preparation, selective PCR amplification, electrophoresis on an automated DNA sequencer and data processing were performed as described previously (Huys & Swings, 1999). Microscale DNA extracts were also used for repetitive sequence-based PCR fingerprinting using the ERIC1R and ERIC2 oligonucleotide primers (ERIC-PCR) (Versalovic *et al.*, 1991). ERIC-PCR was performed twice during independent runs according to the protocol described by Versalovic *et al.* (1994) with the exception that Tris/HCl was used at pH 8.0 and that Red Goldstar Taq polymerase (Eurogentec) was used. PCR products were electrophoresed in 1.5% agarose for 16 h at 2 V cm⁻¹ in 1 × TAE buffer (Versalovic *et al.*, 1994) at 4 °C. ERIC-PCR profiles were visualized after ethidium bromide staining and captured digitally using a CCD camera.

All strains listed in Table 1 and three reference strains of *A. hydrophila* HG1 (LMG 2844^T, LMG 13443 and LMG 13658) were tested for 152 physiological and

biochemical properties as described previously (Huys *et al.*, 1997b). The urocanic acid assimilation test, which was not included in the latter study, was performed according to Hänninen (1994). A total of 30 features was selected for repeated determinations. For all strains under study, the two readings gave identical results, demonstrating the excellent reproducibility of the method. For each BD-2 isolate, antimicrobial susceptibilities were determined for six antimicrobial agents by the disc diffusion method. The following antibiotic discs (Oxoid) were applied using a ST6090 Disc Dispenser (Oxoid): nalidixic acid (30 µg), penicillin (10 µg), ampicillin (25 µg), tetracycline (30 µg), kanamycin (30 µg) and streptomycin (25 µg). Antibio-grams were determined according to the conventional Kirby–Bauer method (Bauer *et al.*, 1966) with the exception that Mueller–Hinton medium was replaced by IS broth and ISA medium (Traub *et al.*, 1998). Isolates were classified into three categories based on the quantitative interpretation criteria recommended by the NCCLS (1993). Four BD-2-type strains were used in a DNA–DNA hybridization study that also included the type strains of all unique *Aeromonas* species. Genomic DNA was prepared using a combination of the protocols of Marmur (1961) and Pitcher *et al.* (1989), as described by Goris *et al.* (1998). Hybridizations were performed using the fluorometric microplate method (Ezaki *et al.*, 1989) with modifications by Goris *et al.* (1998) at an optimal renaturation temperature of 45 °C in the presence of 50% formamide.

In order to validate previously reported AFLP identification data obtained with the radioactive methodology (Kühn *et al.*, 1997) and to update the laboratory database generated from FAFLP fingerprints (Huys & Swings, 1999), a subset of BD-2 strains was analysed in a new FAFLP experiment. Comparison of the digitized FAFLP banding patterns with the AEROLIB database (Huys & Swings, 1999) revealed that the BD-2 isolates represented a homogeneous cluster that was clearly separated from all other *Aeromonas* taxa. A simplified version of this numerical analysis is shown in Fig. 1. The closest taxonomic neighbours of this cluster were *A. hydrophila* HG1 strains, which joined the *Aeromonas* BD-2 cluster at a correlation level of 36%. Given that *Aeromonas* species are usually delineated at a correlation level of 35% or less in numerical analysis of FAFLP fingerprints (Huys & Swings, 1999), the relative genotypic position of *Aeromonas* BD-2 in Fig. 1 did not allow us to predict whether the type BD-2 group deserves species or subspecies rank in the genus *Aeromonas*. Likewise, visual comparison of ERIC-PCR fingerprints allowed five *A. hydrophila* HG1 strains to be distinguished as a group from 10 type BD-2 isolates in two independent PCR runs. Clear differentiation between the two groups was possible due to the specific presence of two ERIC-PCR markers with estimated lengths of 2.9 and 3.3 kb in the profiles of *A. hydrophila* HG1 (Fig. 2). Collectively, both FAFLP and ERIC-PCR finger-

Table 3. Phenotypic characteristics for differentiation of the BD-2 group from *A. hydrophila* HG1

Characters are scored as: +, all strains positive; –, all strains negative; (+), all strains positive except strain LMG 19559.

Characteristic	BD-2 group (n = 10)	<i>A. hydrophila</i> HG1 (n = 3)
Utilization of:		
L-Arabinose	–	+*
L-Fucose	–	+
Methyl α -D-mannoside	–	+
Urocanic acid	+	–*
cis-Aconitate	(+)	–
DL-Aspartate	+	–
Caprate	(+)	–
Acid from L-arabinose	–	+*

* Data confirmed in other studies (Abbott *et al.*, 1992; Hänninen, 1994; Kämpfer & Altwegg, 1992).

printing results indicate that group BD-2 is genotypically distinct from *A. hydrophila* HG1. This finding prompted us to perform DNA–DNA hybridizations and an extensive phenotypic characterization to determine the taxonomic location of the ‘*A. hydrophila*-like’ BD-2 group.

The DNA hybridization data presented in Table 2 clearly demonstrate the high relatedness between the three representatives of the ‘*A. hydrophila*-like’ BD-2 group and the type strain LMG 2844^T of *A. hydrophila* HG1 (78–92%) and two other HG1 reference strains, LMG 13443 and LMG 13658 (77–94%). Within the BD-2 strains, the intragroup relatedness ranged from 77 to 94% (Table 2). In congruence with the FAFLP grouping in Fig. 1, these homology data indicate that the BD-2 strains represent a homogeneous group that belongs genotypically to *A. hydrophila* HG1. This relationship was further substantiated by DNA hybridization values of strains LMG 19559, LMG 19562^T and LMG 19564 with the type strains of the 13 other species currently recognized in *Aeromonas*, which revealed that the BD-2 isolates were 31–67% related to other *Aeromonas* species (Table 2).

Following comparison of 152 phenotypic properties, it was found that the 10 *Aeromonas* BD-2 isolates could be distinguished from strains LMG 2844^T, LMG 13443 and LMG 13658 of its closest genotypic neighbour, *A. hydrophila* HG1, by a total of eight tests. The results for *A. hydrophila* HG1 for three of these tests, utilization of L-arabinose and urocanic acid and acid production from L-arabinose (Table 3), have also been confirmed uniformly in previous studies that included an extended panel of HG1 reference strains (Abbott *et al.*, 1992; Hänninen, 1994; Kämpfer & Altwegg, 1992). Hence, these three characteristics should be considered as major key tests that allow the differentiation of *Aeromonas* BD-2 from *A. hydrophila* HG1. The other

five tests that differentiated the *Aeromonas* BD-2 group from the three *A. hydrophila* HG1 strains were utilization of cis-aconitate, DL-aspartate, caprate, L-fucose and α -methyl D-mannoside (Table 3). Although no studies were found in the *Aeromonas* literature that reported data on the assimilation of these substrates by *A. hydrophila* HG1, it is expected that several of these tests may provide additional key characteristics to facilitate the phenotypic differentiation of the latter taxon from *Aeromonas* group BD-2. In addition to the differential tests, the BD-2 group also shared a number of biochemical properties with *Aeromonas* HG1, i.e. positive test results for lysine decarboxylase, arginine dihydrolase, Voges–Proskauer reaction and acid from sucrose (Table 4; Abbott *et al.*, 1992). In addition, the arabinose-negative BD-2 group and *A. hydrophila* HG1 both utilized DL-lactate and failed to form acid from sorbitol and rhamnose, which are useful key characteristics to separate these two taxa from other members of the *A. hydrophila* complex, i.e. *Aeromonas bestiarum* HG2 and the currently unnamed HG3 (Table 4; Hänninen, 1994). Notably, Renaud *et al.* (1988) also previously isolated an atypical group of arabinose-negative *A. hydrophila* strains that, in contrast with all members of the BD-2 group (Table 4), failed to assimilate DL-lactate.

Based on the clear genotypic (Figs 1 and 2) and phenotypic (Table 3) differences observed between the BD-2 group and the strains described as *A. hydrophila* HG1 and given the high genomic relatedness between the two groups on the basis of DNA–DNA hybridization results (Table 2), the data presented in this study indicate that the BD-2 group represents a subspecies of *A. hydrophila* HG1. According to the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and the DSMZ Bacterial Nomenclature Up-to-date site (<http://www.gbf-braunschweig.de/DSMZ/bactnom/>), three subspecies are recognized in the species *A. hydrophila*, subsp. *hydrophila*, subsp. *anaerogenes* and subsp. *proteolytica*. However, it should be mentioned that this subspeciation is still based on the *Aeromonas* taxonomy proposed by Schubert (1974) and that none of these subspecies names have been used since the description of the genus *Aeromonas* of Popoff (1984). In the latter chapter, Popoff (1984) clearly outlined that *A. hydrophila* subsp. *proteolytica* (Schubert 1969) is phenotypically and genotypically different from other aeromonads, leading to its inclusion in the genus *Vibrio* as *Vibrio proteolyticus* in the same edition of *Bergey's Manual* (Baumann *et al.*, 1984). Based on a previous DNA–DNA hybridization study (Popoff *et al.*, 1981), *A. hydrophila* subsp. *anaerogenes* was found to comprise three DNA hybridization groups that were subsequently placed in *Aeromonas caviae* (groups 239 and 545; Popoff *et al.*, 1981) and in *Aeromonas eucrenophila* (strain 546; Schubert & Hegazi, 1988). Finally, Popoff (1984) suggested that *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980) deserved species status, leading to the proposal of the species

Table 4. Key tests for the phenotypic differentiation of *A. hydrophila* subsp. *dhakensis* from other mesophilic *Aeromonas* species

Data were taken from Altwegg & Lüthy-Hottenstein (1991), Abbott *et al.* (1992) and Kämpfer & Altwegg (1992) unless otherwise indicated. Taxa are identified as: 1, *A. hydrophila* subsp. *dhakensis* (data from this study); 2, *A. hydrophila* subsp. *hydrophila* (this study; Abbott *et al.*, 1992; Kämpfer & Altwegg, 1992; Hänninen, 1994); 3, *A. bestiarum* (Abbott *et al.*, 1992; Kämpfer & Altwegg, 1992; Hänninen, 1994); 4, *A. caviae*; 5, *A. media*; 6, *A. eucrenophila* (Huys *et al.*, 1997a); 7, *A. sobria*; 8, *A. veronii* biovar *sobria*; 9, *A. veronii* biovar *veronii*; 10, *A. jandaei*; 11, *A. encheleia* (Huys *et al.*, 1997a); 12, *A. schubertii*; 13, *A. trota*; 14, *Aeromonas allosaccharophila* (Martinez-Murcia *et al.*, 1992); 15, *A. popoffii* (Huys *et al.*, 1997b). Characters are scored as: +, $\geq 85\%$ of strains positive; –, $\geq 85\%$ of strains negative; v+, 50–85% of strains positive; v–, 50–85% of strains negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Utilization of DL-lactate	+	+	–	v+	+	–	v+	–	–	+	–	v+	ND	ND	+
Acid from:															
Salicin	+	+	v–	+	v+	+	–	–	+	–	+	–	–	–	–
D-Cellobiose	–	v–	v–	v+	+	+	+	v–	v+	–	–	–	+	+	–
L-Arabinose	–	+	+	+	+	v+	–	–	–	–	v–	–	–	v+	v–
D-Sucrose	+	+	+	+	+	–	+	+	+	–	v–	–	v–	+	–
Decarboxylation of:															
Ornithine	–	–	–	–	–	–	–	–	+	–	–	–	–	v–	–
Lysine	+	+	+	–	–	–	+	+	+	+	–	v+	+	+	–
Arginine dihydrolase	+	+	+	+	+	+	–	+	–	+	+	+	+	v+	+

name *A. hydrophila* with strain ATCC 7966^T as the type strain. However, since the taxon name *A. hydrophila* subsp. *hydrophila* still formally exists and represents strains commonly referred to as *A. hydrophila* HG1 (Carnahan & Altwegg, 1996), the authors propose to extend its description in relation to the creation of a new subspecies to accommodate the BD-2 group. The name *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov. is proposed for this new taxon.

The two subspecies of the mesophilic species *A. hydrophila* can be readily distinguished from the five currently known subspecies of *Aeromonas salmonicida* (subspecies *salmonicida*, *masoucida*, *achromogenes*, *smithia* and *pectinolytica*) by their motility, their ability to grow at 37 °C and/or their inability to produce a brown, water-soluble pigment (Pavan *et al.*, 2000). As indicated in Table 4, the two subspecies of *A. hydrophila* can be differentiated from the other *Aeromonas* species in two or more test results using the following eight key features: utilization of DL-lactate, acid from salicin, D-cellobiose, L-arabinose and D-sucrose, decarboxylation of ornithine and lysine and activity of arginine dihydrolase.

As with *A. hydrophila* subsp. *hydrophila* (formerly *A. hydrophila* HG1), the clinical significance of *A. hydrophila* subsp. *dhakensis* is unclear. The majority of the BD-2 isolates were recovered from patients with diarrhoea (23 isolates) and only one isolate was found in a healthy control (LMG 19560) and one isolate in a pond (not included in this study) (Kühn *et al.*, 1997). In addition, most patient strains, including the control isolate LMG 19560, displayed strong cytotoxic and haemolytic effects (Table 1). However, because many patients from whom BD-2 strains were isolated had

co-infections with established enteric pathogens such as *Campylobacter jejuni*, *Salmonella* spp. and *Shigella* spp., the primary pathogenic role of BD-2 strains can not yet be assessed with certainty (Kühn *et al.*, 1997). In Bangladesh, gastrointestinal infections with multiple pathogens are common, which suggests the possibility of synergism among pathogens to produce diarrhoea (Albert *et al.*, 1999). We therefore propose that the pathogenicity of *A. hydrophila* subsp. *dhakensis* should be further elucidated, including a prevalence study of the recently characterized *Aeromonas* enterotoxin genes *alt*, *ast* and *act* (Albert *et al.*, 2000).

Description of *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov.

Aeromonas hydrophila subsp. *dhakensis* (dha.ken'sis. N.L. adj. *dhakensis* of Dhaka, Bangladesh)

Includes the 10 strains listed in Table 1. All child patients from which these strains were isolated lived in the vicinity of Dhaka and were treated at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), in Dhaka. Strains display the following characteristics typical of the genus *Aeromonas*: Gram-negative, straight, motile rods, chemo-organotrophic with both oxidative and fermentative metabolism, cytochrome oxidase- and catalase-positive, reduction of nitrate to nitrite without the production of gas and resistant to the vibriostatic agent O/129. Optimal growth occurs after 24 h at 28 °C on TSA medium, but all strains also grow at 42 °C after 24–48 h. No brown, water-soluble pigment is produced on TSA medium. Arginine dihydrolase-, lysine decarboxylase-, indole- and Voges-Proskauer-

positive. No production of urease, tryptophan deaminase, ornithine decarboxylase or H₂S. The following substrates are used as sole carbon and energy sources: acetate, *N*-acetyl-D-glucosamine, *cis*-aconitate (except strain LMG 19559), L-alanine, arbutin, L-arginine, DL-aspartate, butyrate, caprate (except strain LMG 19559), D-fructose, fumarate, D-galactose, D-glucuronate, D-glucosamine, D-glucose, L-glutamine, glycerol, L-glycine, L-histidine, isobutyrate, DL-lactate, lactose, DL-malate, D-mannitol, D-mannose, D-maltose, L-proline, putrescine, pyruvate, D-ribose, salicin, spermidine, succinate, L-serine, D-sucrose, D-trehalose and urocanic acid. None of the strains uses *trans*-aconitate, adipate, adonitol, 4-aminobutyrate, azelate, betaine, cadaverine, D-cellobiose, L-citrulline, erythritol, D-fucose, L-fucose, D-glucosaminic acid, D-glucuronate, glutarate, 3-hydroxybenzoate, 4-hydroxybenzoate, inositol, isovalerate, itaconate, L-leucine, L-lysine, maltitol, α -D-melibiose, mesaconate, methyl α -D-mannoside, phenylacetate, L-phenylalanine, D-raffinose, L-rhamnose, sorbate, D-sorbitol, spermine, suberate, L-tryptophan, L-valine, D-xylitol or D-xylose. Acid is uniformly produced from D-galactose, D-glucose, D-maltose, D-mannitol, D-mannose, salicin, D-sucrose and D-trehalose, but not from adonitol, L-arabinose, D-arabitol, D-cellobiose, dulcitol, erythritol, inositol, α -D-melibiose, methyl D-glucoside, lactose, D-raffinose, L-rhamnose, D-sorbitol or D-xylose. All strains hydrolyse the following substrates: L-alanine *p*-nitroanilide (pNA), 2-deoxythymidine-5'-*p*-nitrophenyl (pNP) phosphate, aesculin, gelatin, bis-pNP phosphate, *ortho*-nitrophenyl β -D-galactopyranoside, pNP β -D-glucopyranoside, pNP phenylphosphonate, pNP phosphorylcholine and L-proline pNA. None of the strains is able to hydrolyse L-glutamate- γ -3-carboxy pNA, pNP α -D-glucopyranoside or pNP β -D-glucuronide. All strains are resistant to ampicillin and penicillin but sensitive to kanamycin. For resistance to nalidixic acid, streptomycin and tetracycline, resistant (including strains LMG 19559, LMG 19561, LMG 19564, LMG 19566 and LMG 19567) and susceptible (LMG 19558, LMG 19560, LMG 19562^T, LMG 19563 and LMG 19565) subgroups were observed. Isolated from stool samples of children (under the age of five) suffering from diarrhoea. One strain (LMG 19560) originated from a healthy child (Table 1). All isolates except strain LMG 19561 are strongly β -haemolytic to sheep erythrocytes. Eight of the ten strains produce high ($\geq 1/256$) cytotoxin titres (Table 1). The type strain is strain LMG 19562^T (= CCUG 45377^T).

Emended description of *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980)

As mentioned above, the name *A. hydrophila* subsp. *hydrophila* was included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) but was assigned species rank as *A. hydrophila* by Popoff (1984). As a result of the taxonomic allocation of the biochemically distinct BD-2 group to *A. hydrophila*,

the description of *A. hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (Approved Lists 1980) needs to be extended somewhat. The following description of the latter taxon is based on phenotypic data determined from the three HG1 strains included in this study and on data reported previously for *A. hydrophila* HG1 by Abbott *et al.* (1992), Kämpfer & Altwegg (1992) and Hänninen (1994), which all included the type strain, ATCC 7966^T (= LMG 2844^T).

Strains display the following characteristics typical of the genus *Aeromonas*: Gram-negative, straight, motile rods, chemo-organotrophic with both oxidative and fermentative metabolism, cytochrome oxidase- and catalase-positive, reduction of nitrate to nitrite without the production of gas and resistant to the vibriostatic agent O/129. Glucose is fermented with production of acid and gas. Optimal growth occurs after 24 h at 28 °C on TSA medium, but all strains also grow at 41 °C after 24–48 h. No brown, water-soluble pigment is produced on TSA medium. Arginine dihydrolase-, lysine decarboxylase-, indole-, aesculin hydrolysis- and Voges-Proskauer-positive. No production of urease, tryptophan deaminase, ornithine decarboxylase or H₂S. The following substrates are used as sole carbon and energy sources: *N*-acetyl β -D-galactosamine, L-alanine, L-arabinose, *p*-arbutin, DL-lactate, D-mannitol, putrescine, D-serine, salicin and D-sucrose. Strains do not use D-cellobiose, DL-isocitrate, β -alanine, 4-aminobutyrate or urocanic acid. Acid is produced from L-arabinose, D-mannitol, salicin, and D-sucrose, but not from D-cellobiose, lactose, D-rhamnose or D-sorbitol. See Table 3 for additional phenotypic markers for differentiation from *A. hydrophila* subsp. *dhakensis*. Isolated from humans, animals, fish and fresh water. Strong β -haemolytic activity on TSA supplemented with 5% sheep blood. The type strain is strain ATCC 7966^T (= LMG 2844^T).

Acknowledgements

G.H. is a post-doctoral fellow of the Fund for Scientific Research – Flanders (Belgium) (FWO–Vlaanderen).

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