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Escherichia albertii sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children

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The taxonomic position of a group of five D-sorbitol- and lactose-negative enterobacterial isolates recovered from diarrhoeal stools of children at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), was investigated by DNA–DNA hybridization, phenotypic characterization and 16S rDNA sequencing. These strains were originally identified as '*Hafnia alvei*-like' with the API 20E system but, in fact, show more phenotypic and genotypic resemblance to members of the genus *Escherichia*. By 16S rDNA sequencing, one representative strain of the ICDDR,B group was shown to be closely affiliated to the genera *Escherichia* and *Shigella*. Using the fluorimetric microplate hybridization method, the diarrhoeagenic ICDDR,B isolates were found to constitute a homogeneous taxon (⩾82 % internal DNA relatedness), with the closest affiliation to the type strains of *Escherichia coli* (55–64 %) and *Shigella flexneri* (54–60 %). The DNA–DNA hybridization levels were much lower with members of other described *Escherichia* species (16–45 %) and with the type strain of *H. alvei* (9–17 %). The G+C content of the ICDDR,B strains ranged from 50·5 to 50·7 mol%. Together with the diagnostic characteristics reported previously, including the presence of the *eaeA* gene of enteropathogenic *E. coli* and of the *E. coli* and *Shigella*-specific *phoE* gene, it is concluded that the ICDDR,B strains represent a novel taxon in the genus *Escherichia*, for which the name *Escherichia albertii* sp. nov. is proposed. Its type strain is Albert 19982^T (=LMG 20976^T=CCUG 46494^T).

In a previous study, Janda *et al.* (1999) described a group of five diarrhoeagenic strains that were allocated to the genus *Escherichia* on the basis of phenotypic and genotypic evidence. These isolates all originated from stool specimens of children with diarrhoeal illness and were recovered by workers at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh (Albert *et al.*, 1991b). Initially, the five ICDDR,B stool isolates were assigned to the species *Hafnia alvei* by the API 20E system. However, Albert *et al.* (1992b) later discovered that all of these strains, tentatively identified as *H. alvei*, possessed the attaching-and-effacing *eaeA* gene typical of enteropathogenic *Escherichia coli*. In addition, partial 16S rRNA sequence analysis of *eaeA*-positive and *eaeA*-negative *H. alvei* isolates revealed a relatively low level of similarity (92 %; a partial 16S rDNA sequence of ICDDR,B strain LMG 20973 was submitted to the EMBL database under accession no. Z47360), indicating that the ICDDR,B isolates were probably misidentified as *H. alvei* (Ridell *et al.*, 1995). Likewise, Ismaili *et al.* (1996) reported a number of striking phenotypic and genotypic differences between Canadian *H. alvei* isolates and the ICDDR,B stool isolate 19982 based on *eaeA*-induced cellular responses and on outer-membrane protein (OMP), PFGE and plasmid profile analysis. More recently, conventional biochemical characterization, determination of susceptibility to the antibiotic cephalothin and to a *Hafnia*-specific phage, PCR-based detection of the OMP gene *phoE*, PFGE and partial 16S rDNA sequencing have further supported the notion that the diarrhoeagenic ICDDR,B isolates actually belong to the genus *Escherichia* (Janda *et al.*, 1999, 2002). It was concluded that these isolates either represent an unusual biotype of *E. coli* or constitute a novel *Escherichia* species. In the present study, DNA–DNA hybridizations, additional phenotypic characterization and 16S rDNA sequencing were performed in order to elucidate the taxonomic position of the diarrhoeagenic *Escherichia* strains.

The five ICDDR,B strains [LMG 20972 (=Albert 9194), LMG 20973 (=Albert 10457), LMG 20974 (=Albert 10790),

Abbreviation: ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of ICDDR,B strain LMG 20976^T is AJ508775.

LMG 20975 (=Albert 12502) and LMG 20976^T (=Albert 19982^T)] all originated from faeces of diarrhoeal children (<5 years old) and were isolated in 1990–1991 in Dhaka, Bangladesh (Albert *et al.*, 1991b; Ridell *et al.*, 1995; Janda *et al.*, 1999, 2002). Type strains of *Escherichia* species, *H. alvei* and *Shigella flexneri* were obtained from the BCCM^{TM}/LMG Bacteria Collection, Ghent University, Belgium (http://www.belspo.be/bccm/). 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 37 °C for 24 h. In addition to previous phenotypic studies (Ridell *et al.*, 1995; Janda *et al.*, 1999, 2002), the five ICDDR,B isolates were further characterized biochemically with the API 50 CH system (bioMérieux) according to the manufacturer's instructions. Antimicrobial susceptibilities to penicillin (10 μg), ampicillin (25 μg), tetracycline (30 μg), kanamycin (30 μg), streptomycin (25 μg) and chloramphenicol (30 μg) were determined by the disc diffusion method using Oxoid discs according to the conventional Kirby–Bauer method (Bauer *et al.*, 1966). Isolates were classified as resistant, intermediate or susceptible on the basis of the quantitative interpretation criteria recommended by the NCCLS (1993). For determination of the G+C content, DNA was enzymically degraded into nucleosides according to the protocol of Mesbah *et al.* (1989). The mixture was then separated by HPLC using a Waters SymmetryShield C8 column thermostatted at 37 °C. The solvent used was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. For the purpose of DNA–DNA hybridizations, 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 fluorimetric microplate method (Ezaki *et al.*, 1989) with modifications by Goris *et al.* (1998) at an optimal renaturation temperature of 40 °C in the presence of 50 % formamide. The complete 16S rDNA sequence of strain LMG 20976^T was determined as described previously (Huys *et al.*, 2001) using an ABI PRISM 3100 Genetic Analyzer.

As a result of the taxonomic data reported by Ridell *et al.* (1995) and Janda *et al.* (1999), the initial placement of the *eaeA*-positive diarrhoeagenic ICDDR,B isolates in the genus *Hafnia* (Albert *et al.*, 1992b) was called seriously into question. Although a great deal of phenotypic and genotypic evidence has been put forward in favour of the inclusion of these strains in the genus *Escherichia* (Janda *et al.*, 1999, 2002), a final conclusion on their taxonomic status was hampered by the lack of a decisive DNA–DNA hybridization study. As determined with the fluorimetric microplate method, the five *eaeA*-positive diarrhoeagenic ICDDR,B isolates included in the present study were found to constitute a highly homogeneous group, exhibiting internal relatedness between 82 and 100 % (Table 1). In addition, the five isolates under study displayed a very narrow range of G+C content, 50·5–50·7 mol%. These findings clearly demonstrate the genotypic homogeneity among the ICDDR,B isolates as reflected previously by their highly similar RAPD-PCR profiles and identical 353-bp fragments of the 16S rRNA genes (Ridell *et al.*, 1995) and typical PFGE profiles (Janda *et al.*, 1999).

In a second DNA–DNA hybridization study, two *eaeA*-positive diarrhoeagenic ICDDR,B isolates (LMG 20974 and LMG 20976^T) were cross-hybridized with the type strains of the five species currently recognized in the genus *Escherichia*, *E. coli*, *Escherichia blattae*, *Escherichia fergusonii*,

Table 1. DNA relatedness between the ICDDR,B group and type strains of other enterobacterial species

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 %. –, Not tested.

Strain (unlabelled DNA)	Source of labelled DNA											
	1	2	3	4	5	6	7	8	9	10	11	12
ICDDR,B strains												
1. LMG 20976^T	100	98	102	101	82	55	15	42	20	22	54	17
2. LMG 20974	96	100	98	101	–	59	14	35	20	23	57	17
3. LMG 20972	91	93	100	99	–	–	–	–	–	–	–	–
4. LMG 20973	85	95	94	100	–	–	–	–	–	–	–	–
5. LMG 20975	89	–	–	–	100	–	–	–	–	–	–	–
6. *E. coli* LMG 2092^T	64	61	–	–	–	100	18	63	–	–	75	–
7. *E. blattae* LMG 3030^T	17	18	–	–	–	20	100	18	–	–	–	–
8. *E. fergusonii* LMG 7866^T	44	45	–	–	–	59	17	100	–	–	–	–
9. *E. hermanii* LMG 7867^T	18	20	–	–	–	–	–	–	100	28	–	14
10. *E. vulneris* LMG 7868^T	17	16	–	–	–	–	–	–	27	100	–	10
11. *S. flexneri* LMG 10472^T	60	54	–	–	–	71	–	–	–	–	100	–
12. *H. alvei* LMG 10392^T	10	9	–	–	–	–	–	–	10	11	–	100

Escherichia hermanii and *Escherichia vulneris*. As somewhat expected from their phenotypic relatedness, strains LMG 20974 and LMG 20976^T displayed the highest DNA relatedness values, 55-64 %, to the type strain of *E. coli*, LMG 2092^T (=ATCC 11775^T) (Table 1). A comparable range of genomic relatedness (54–60 %) was also found with the type strain of *S. flexneri* (LMG 10472^T=ATCC 12022^T =CDC 3591-52^T), which is reflective of the fact that representatives of the genus *Shigella* cannot be differentiated from *E. coli* on the basis of DNA–DNA hybridization (Brenner *et al.*, 1973; Watanabe & Okamura, 1992). The DNA relatedness of the two ICDDR,B strains to the type strains of other *Escherichia* species was much lower, ranging from 14 to 45 % (Table 1). The inclusion of *eaeA*-positive ICDDR,B isolates in *H. alvei*, as initially suggested (Albert *et al.*, 1991b, 1992b), was definitely ruled out by the finding that the type strain of the latter taxon, strain LMG 10392^T (=ATCC 13337^T), was only 9–17 % related to ICDDR,B strains LMG 20974 and LMG 20976^T (Table 1). The DNA–DNA hybridization values obtained in this study clearly indicate that the *eaeA*-positive diarrhoeagenic ICDDR,B isolates constitute a homogeneous taxon in the genus *Escherichia* that is most closely affiliated with, but still well below 70 % related (concordant with the recommendations of Wayne *et al.*, 1987) to, *E. coli* and *S. flexneri*. This conclusion is further supported by previously reported genotypic diagnostic markers, including the presence of the *eaeA* gene of enteropathogenic *E. coli* and of the *E. coli* and *Shigella*-specific *phoE* gene (Albert *et al.*, 1992b; Janda *et al.*, 1999; Ridell *et al.*, 1995).

Pairwise comparison of the 16S rDNA sequence of ICDDR, B strain LMG 20976^T with those of the neighbouring taxa retrieved from the EMBL database (http://srs.ebi.ac.uk) revealed the following similarity values: 99·3 % with *Shigella sonnei* LMG 10473^T (=ATCC 25931^T) (accession no. X96964) and *S. flexneri* ATCC 29903^T (X96963), 98·3 % with *E. coli* LMG 2092^T (=ATCC 11775^T) (X80725), 97·8 % with *E. vulneris* LMG 7868^T (=ATCC 33821^T) (X80734) and 93·5 % with *H. alvei* LMG 10392^T (=ATCC 13337^T) (M59155). Taken together, these results reinforce the conclusion that the ICDDR,B group does not belong to *H. alvei* and confirm its close phylogenetic affiliation to the genera *Escherichia* and *Shigella*.

Collectively, the results of API 50CH characterization obtained in this study agreed well with the phenotypic data reported previously on *eaeA*-positive diarrhoeagenic ICDDR,B isolates (Ridell *et al.*, 1995; Janda *et al.*, 1999, 2002) and clearly indicate that this novel group of *Escherichia* strains are also phenotypically significantly different from their closest enterobacterial relatives. The ICDDR,B strains can be clearly distinguished from *E. coli* by negative reactions for indole and for fermentation of lactose and D-sorbitol and by the inability to produce β-D-glucuronidase. In addition, the novel group of *Escherichia* strains can be separated from the other described *Escherichia* species by at least two phenotypic characteristics, including fermentation of D-xylose, which is negative for the ICDDR, B group (Table 2). Although biochemical distinction from *Shigella* isolates may be confusing, the ICDDR,B strains can be distinguished from shigellae by gas production from D-glucose and the ability to produce lysine decarboxylase. By serotyping, the ICDDR,B strains were found to be non-reactive against antisera specific to *Shigella boydii* serotypes 13 and 14 (J. M. Janda, unpublished data). On a genetic basis, it was previously reported that the ICDDR,B strains do not possess the invasion antigen H (*ipaH*), which is typically present in shigellae and enteroinvasive *E. coli* (Janda *et al.*, 1999). Clear phenotypic distinction from *H. alvei* strains can be made on the basis of acetate assimilation, negative reactions for Voges–Proskauer and growth in KCN broth and resistance to the *Hafnia*-specific bacteriophage 1672.

Description of *Escherichia albertii* sp. nov.

Escherichia albertii [al.ber′ti.i. N.L. gen. n. *albertii* of Albert, named after M. John Albert, a microbiologist previously associated with the ICDDR,B, who first described these strains as a group of *Hafnia*-like diarrhoeagenic isolates in the early 1990s and who also contributed greatly to the study of other diarrhoeagenic bacteria (Albert, 1994; Albert *et al.*, 1991a, 1992a, 2000)].

This description is based on data reported previously by Ridell *et al.* (1995) and Janda *et al.* (1999, 2002) and on API 50CH profiling performed in the course of this study.

Table 2. Key tests for phenotypic differentiation between *Escherichia albertii* sp. nov. and other *Escherichia* species

Species are indicated as: 1, *E. albertii*; 2, *E. coli*; 3, *E. blattae*; 4, *E. fergusonii*; 5, *E. hermanii*; 6, *E. vulneris*. Data for *E. albertii* from this study and from Janda *et al.* (1999); data for other species from Farmer *et al.* (1985). Characters are scored as: +, ⩾85 % of strains positive; −, ⩾85 % of strains negative; V+, 50–85 % of strains positive; V−, 50–85 % of strains negative.

Key test	1	2	3	4	5	6
Indole	−	+	−	+	+	−
Lysine decarboxylase	+	+	+	+	−	+
Ornithine decarboxylase	+	V+	+	+	+	−
Growth in KCN	−	−	−	−	+	−
Fermentation of:						
Lactose	−	+	−	−	V−	−
D-Mannitol	+	+	−	+	+	+
Adonitol	−	−	−	+	−	−
D-Sorbitol	−	+	−	−	−	−
Raffinose	−	V+	−	−	V−	+
L-Rhamnose	−	V+	+	+	+	+
D-Xylose	−	+	+	+	+	+
Cellobiose	−	−	−	+	+	+
D-Arabitol	−	−	−	+	−	−
Utilization of acetate	+	+	−	+	V+	V−

All five isolates display the following characteristics typical of the genus *Escherichia*: cells are Gram-negative, medium to long rods, chemo-organotrophic with both oxidative and fermentative metabolism, cytochrome oxidase-negative and catalase-positive and acid and gas are produced from D-glucose. Growth occurs after 24 h at 35–37 °C on TSA medium. Cells are non-motile at 35 °C. Positive for lysine and ornithine decarboxylases, methyl red reaction and nitrate reduction but negative for arginine dihydrolase, indole, DNase, urease, gelatinase and Voges–Proskauer reaction. No growth in KCN broth. Acetate but not citrate or malonate are used as carbon sources. 3-Hydroxybenzoate but not 2-ketogluconate or histidine is assimilated. β-D-Glucuronidase is not produced. Acid is produced from L-arabinose and D-mannitol but not from adonitol, amygdalin, D-arabitol, D-cellobiose, dulcitol, erythritol, inositol, lactose, α-D-melibiose, methyl D-glucoside, D-raffinose, L-rhamnose, salicin, D-sorbitol, D-sucrose or D-xylose. Fermentation of D-maltose and D-trehalose is variable (negative for strains LMG 20972 and LMG 20974). By disc diffusion testing, resistant to tetracycline and penicillin but susceptible to chloramphenicol, kanamycin and ampicillin (except strain LMG 20974). All strains are partially or completely susceptible to cephalothin by the E-test. The G+C content is 50·5–50·7 mol%.

The type strain, strain Albert 19982^T (=LMG 20976^T =CCUG 46494^T), was isolated from the stool specimen of a diarrhoeal child in Bangladesh. The G+C content of this strain is 50·6 mol%.

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