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NOTE

DNA–DNA reassociation and phenotypic data indicate synonymy between *Aeromonas enteropelogenes* Schubert *et al.* 1990 and *Aeromonas trota* Carnahan *et al.* 1991

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Mainly on the basis of phylogenetic and genotypic evidence, it has been suggested previously that the species *Aeromonas enteropelogenes* Schubert *et al.* 1990 is identical to the species *Aeromonas trota* Carnahan *et al.* 1991. Probably because the description of *A. enteropelogenes* preceded the proposal of *A. trota* by only a few months, DNA–DNA hybridizations were never performed between representative strains of these two taxa. In the present study, new DNA–DNA hybridizations between the type strain of *A. enteropelogenes*, LMG 12646^T (= DSM 6394^T), and reference strains of *A. trota*, including its type strain LMG 12223^T (= ATCC 49657^T), showed a genomic relatedness of 81–99%. In addition, phenotypic characterization revealed that the two type strains exhibited identical API 20E and API 50CHE biochemical profiles and were both susceptible to ampicillin and carbenicillin. Collectively, our new DNA reassociation and phenotypic data confirm previous taxonomic data that indicate that the taxa *A. enteropelogenes* and *A. trota* are synonymous members of the same *Aeromonas* species. Although the species name *A. enteropelogenes* has nomenclatural priority, the authors would like to discourage the use of this name because the name *A. trota* has been cited much more frequently. The preferential use of *A. trota* in future publications may be the best option to avoid ambiguity in the description of ampicillin-susceptible aeromonads and to secure nomenclatural continuity in *Aeromonas* literature.

Keywords: *Aeromonas enteropelogenes*, *Aeromonas trota*, DNA–DNA hybridizations

Until the 1990s, it was generally assumed that all members of the genus *Aeromonas* were uniformly resistant to ampicillin and carbenicillin. Because ampicillin resistance is encoded chromosomally in *Aeromonas*, this property is an ideal stable discriminative feature for the selective isolation of aeromonads, e.g. by using ampicillin/dextrin agar (Havelaar *et al.*, 1987). In 1991, however, the use of ampicillin-containing media for screening faecal samples was seriously questioned by the description of *Aeromonas trota* as the first ampicillin-susceptible *Aeromonas* species (Carnahan *et al.*, 1991). The majority of the strains belonging to *A. trota* were isolated from faecal specimens collected in southern and south-eastern Asia and were initially considered as an *Aeromonas sobria*-like group. A few months before the publication of the *A. trota* proposal, Schubert *et al.* (1990) proposed the name *Aeromonas enteropelogenes* for a psychrophilic group of *A. sobria*-like organisms, all of which were recovered from human stools in India. Although originally described as two different species, phylogenetic evidence presented by Collins *et al.* (1993) led to the conclusion that *A. enteropelogenes* and *A. trota* were identical, since the 16S rRNA sequences of their type strains displayed 100% similarity. The latter authors suggested that additional DNA–DNA hybridization studies should be performed in order to determine conclusively the genuine taxonomic relatedness between these species but, to date, these data are still not available. In subsequent studies, fatty acid analysis (G. Huys, unpublished results) and amplified fragment length polymorphism analysis (Huys *et al.*,

Table 1. DNA–DNA relatedness between *A. enteropelogenes* and *A. trota* reference strains

Results are mean percentages from four determinations. Reciprocal hybridizations showed a maximum standard deviation of ±6% whereas repeated experiments exhibited a maximum standard deviation of ±3%.

Strain	DNA hybridization with labelled reference DNA from strain:						
	1	2	3	4	5	6	7
1. *A. enteropelogenes* LMG 12646^T	100	86	84	89	81	47	44
2. *A. trota* LMG 12223^T	98	100	95	94	92	49	40
3. *A. trota* LMG 13080	96	92	100	91	82		
4. *A. trota* LMG 13081	99	89	95	100	91		
5. *A. trota* LMG 13082	93	90	91	90	100		
6. *A. caviae* LMG 3775^T	46	43					
7. *A. sobria* LMG 3783^T	48	46					

1996; Huys & Swings, 1999) further supported the view that *A. enteropelogenes* and *A. trota* cannot be separated on phenotypic or genotypic grounds. In this context, the present study was undertaken to provide decisive DNA–DNA reassociation data for *A. trota* and *A. enteropelogenes* and to assess their phenotypic and antibiotic profiles using a uniform methodology for all strains included.

The following type or reference strains were obtained from the BCCM/LMG Bacteria Collection: *A. enteropelogenes* LMG 12646^T (= ATCC 49803^T = DSM 6394^T = Sanyal J11^T), *A. trota* LMG 12223^T (= ATCC 49657^T), LMG 13080 (= ATCC 49659), LMG 13081 (= ATCC 49660) and LMG 13082 (= ATCC 49658), *Aeromonas caviae* LMG 3775^T (= ATCC 15468^T) and *A. sobria* LMG 3783^T (= CIP 74.33^T). Additional information on these strains can be found in an earlier paper (Huys *et al.*, 1996). Strains were cultured aerobically on Trypticase soy agar containing 3% (w/v) Trypticase soy broth (BBL) and 1·5% (w/v) bacteriological agar no. 1 (Oxoid) at 28 °C for 24 h. *A. enteropelogenes* ($n = 1$) and *A. trota* ($n = 4$) strains were characterized biochemically using API 20E and API 50CHE microbial identification strips according to the manufacturer's instructions (bioMérieux). For each isolate, antimicrobial susceptibilities were assessed for six agents using the disc diffusion method (Bauer *et al.*, 1966) with the modification that Mueller–Hinton medium was replaced by Iso-Sensitest agar (medium CM471, Oxoid) and IS broth (medium CM473, Oxoid) and that strains were incubated at 28 °C. The following antibiotic discs (Oxoid) were applied using an ST6090 disc dispenser (Oxoid): ampicillin (25 μg), carbenicillin (100 μg), kanamycin (25 μg), tetracycline (30 μg), rifampicin (30 μg) and nalidixic acid (30 μg). Isolates were classified based on the quantitative interpretation criteria recommended by the NCCLS (National Committee for Clinical Laboratory Standards, 1993). For the DNA–DNA reassociation study, total genomic DNA was prepared using a combination of the protocols of Marmur (1961) and Pitcher *et al.* (1989) as described previously (Goris *et al.*, 1998). Hybridization experiments 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 50% formamide.

Despite the vast amount of phylogenetic, genotypic and phenotypic information generated so far regarding the taxonomic relationship between *A. trota* and *A. enteropelogenes* (Collins *et al.*, 1993; Huys *et al.*, 1996; G. Huys, unpublished results), a conclusive DNA–DNA reassociation study including representative strains of these two species has never been performed. In this regard, however, it should be mentioned again that the descriptions of the two species were published within a few months of each other, obviously within too short a period to allow cross-referencing. In contrast to the proposal of *A. trota* (Carnahan *et al.*, 1991), the description of *A. enteropelogenes* (Schubert *et al.*, 1990) was based on a rather limited phenotypic and DNA–DNA hybridization study. In 1990, at least eight *Aeromonas* species were validated, but only three of these were included as reference taxa in the latter proposal. Similar to the 16S rRNA sequencing study of Collins *et al.* (1993), the current investigation included just the type strain of *A. enteropelogenes*, LMG 12646^T, which was received as strain DSM 6394^T and which is equivalent to strain Sanyal J11^T originally described by Schubert *et al.* (1990). Together with the type strain and three reference strains of *A. trota*, strain LMG 12646^T was included in a DNA–DNA hybridization study that demonstrated that the two taxa were very highly related, as shown by a DNA hybridization value of 86–98% between the type strains, LMG 12223^T and LMG 12646^T, and by an overall range of DNA relatedness of 81–99% among strains of the two taxa (Table 1). In comparison, the type strains of *A. enteropelogenes* and *A. trota* exhibited 40–49% relatedness with type strains of the non-

related species *A. caviae* (hybridization group 4) and *A. sobria* (hybridization group 7) (Table 1). Collectively, the new DNA–DNA hybridization data are in perfect congruence with the above-mentioned studies suggesting that the taxa *A. enteropelogenes* and *A. trota* are members of the same genomic *Aeromonas* species.

Phenotypically, the type strain of *A. enteropelogenes* and the type and reference strains of *A. trota* could not be separated by any of the 60 different tests included in the API 20E and API 50CHE systems. All *A. enteropelogenes* and *A. trota* strains included in this study displayed the following phenotypic profile. Positive reactions were found for oxidase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, citrate utilization and indole and gelatinase tests, whereas negative reactions were obtained with ornithine decarboxylase, Voges–Proskauer, production of H_2S, urease and tryptophan deaminase. Acid was produced from the following substrates: *N*-acetyl glucosamine, starch, cellobiose, D-fructose, galactose, D-glucose, gluconate, glycerol, glycogen, maltose, D-mannose, mannitol, ribose and trehalose. No acid was produced from adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, aesculin, D-fucose, L-fucose, β-gentiobiose, 2-ketogluconate, 4-ketogluconate, inositol, inulin, lactose, D-lyxose, melibiose, melezitose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-xyloside, D-raffinose, rhamnose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, D-turanose, xylitol, D-xylose and L-xylose. When comparing only the common tests, our phenotypic results were in good agreement with the characteristics reported in the original descriptions of *A. enteropelogenes* (Schubert *et al.*, 1990) and *A. trota* (Carnahan *et al.*, 1991). Solely on the basis of data from the literature, Carnahan (1993) noted previously that the two species shared similar phenotypic profiles, a finding that is now supported using the same method for all strains included.

The high phenotypic relatedness between *A. enteropelogenes* and *A. trota* was also reflected by their antibiotic susceptibility profiles. According to the NCCLS interpretation criteria, all tested strains were found to be susceptible to ampicillin, carbenicillin, kanamycin, tetracycline, rifampicin and nalidixic acid. The remarkable susceptibility of *A. enteropelogenes* strain LMG 12646^T to ampicillin and carbenicillin, which was not reported in the original description of this species by Schubert *et al.* (1990), agrees with the typical susceptibility profile of *A. trota* (Carnahan *et al.*, 1991) and again illustrates the phenotypic closeness between the two taxa. Recently, the strong need to clarify the taxonomic confusion over the synonymy between *A. trota* and *A. enteropelogenes* was emphasized in the studies of Khan *et al.* (1999) and Delamare *et al.* (2000), in which it was reported that two species within the genus *Aeromonas* (*A. trota* and *A. enteropelogenes*) respectively shared a highly similar aerolysin gene and an unusual tolerance to high salt levels.

In conclusion, the DNA–DNA hybridization data presented in the current study clearly indicate that the named taxa *A. trota* and *A. enteropelogenes* belong to the same genomic species based on the high DNA relatedness determined between their type strains. In addition, phenotypic characterization suggested that these strains could not be separated by a single test out of 60 features and both displayed susceptibility to ampicillin and carbenicillin. Together with previous phylogenetic (Collins *et al.*, 1993) and genotypic (Huys *et al.*, 1996) evidence, these new results prompt the authors to conclude that the species names *A. trota* and *A. enteropelogenes* are subjective synonyms. According to Rule 24b of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), the name *A. enteropelogenes* has priority in the bacteriological literature over the name *A. trota* because it was announced in Validation List no. 38 (Schubert *et al.*, 1991) whereas the latter name was included in the later Validation List no. 40 (Carnahan *et al.*, 1992). On the other hand, searches in international scientific citation databases reveal that the name *A. trota* has so far been used much more frequently than the name *A. enteropelogenes*. A search of the Web of Science citation database (Institute for Scientific Information, Thomson Scientific) in the period following the publication of the two original species descriptions revealed 34 references to *A. trota* but only six to *A. enteropelogenes*. Strikingly, all references that include the name *A. enteropelogenes* cover its suspected synonymy with *A. trota* and do not report unique properties of the organism as such. For this reason, it is our opinion that nomenclatural continuity in *Aeromonas* literature can be most optimally secured when the use of *A. trota* is further promoted to describe ampicillin-susceptible aeromonads. To this end, it is anticipated that other workers may find additional arguments in order to formulate a Request for an Opinion from the Judicial Commission to overrule nomenclatural priority.

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