NOTES

DNA Relatedness among *Aeromonas allosaccharophila* Strains and DNA Hybridization Groups of the Genus *Aeromonas*

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The genomic relatedness among three *Aeromonas allosaccharophila* strains, including the type strain, and other *Aeromonas* type and reference strains that were assigned to DNA hybridization groups was estimated by DNA-DNA hybridization (competition procedure using a membrane method). All *A. allosaccharophila* strains were highly related (70 to 100%) to strains 289^T (= CECT 4199^T) and ATCC 35942. Type strains of other validated *Aeromonas* species, reference strains of DNA groups 8 and 11, and the *Aeromonas* sp. strain ATCC 43946 (enteric group 501) were 0 to 41% related to *A. allosaccharophila* 289^T and ATCC 35942. The G+C content of *A. allosaccharophila* strains were in the range 55.9 to 57.3 mol%. The G+C content of the type strain of this species was 56.9 mol%, a value somewhat lower than that reported in the original description.

Kluyver and van Niel (13) proposed the genus *Aeromonas* to accommodate enteric bacterium-like microorganisms that are motile by means of polar flagella. In the first edition of *Bergey's Manual of Systematic Bacteriology* (2), this genus was included in the family *Vibrionaceae*. At present, the proposal to place the genus *Aeromonas* in the new family *Aeromonadaceae* (5), which was based on molecular genetic data, has been recently validated (11). Moreover, the evidence that the genus *Aeromonas* includes 13 DNA homology groups (4, 7, 9, 21) and the recent description of six new mesophilic *Aeromonas* species derived mainly from these DNA relatedness data (1, 3, 4, 9, 10, 22) have significantly changed Popoff's taxonomy of the genus *Aeromonas* (20). On the other hand, results obtained from 16S ribosomal DNA sequencing studies have reported indistinguishable 16S rRNA gene sequences for well-defined genospecies that are based on DNA-DNA reassociation studies, suggesting a lack of sensitivity of rRNA sequencing in separating very closely related species (16). Recently, Martínez-Murcia and colleagues (17) proposed the new species, *Aeromonas allosaccharophila*, to accommodate mesophilic *Aeromonas* strains which had a unique phenotypic profile (6) and on the basis of 16S rRNA gene sequence analysis represented an unknown genetic line within the genus *Aeromonas* (17). However, DNA hybridization studies were not carried out with the *A. allosaccharophila* strains. Thus, the genetic relatedness of this species within the genus *Aeromonas* was not determined. In the present paper we report the results of a DNA-DNA hybridization study comparing *A. allosaccharophila* 289^T (= CECT 4199^T) and ATCC 35942 with other strains of *A. allosaccharophila* and other *Aeromonas* species, as well as the DNA base composition of all *A. allosaccharophila* strains.

A. allosaccharophila strains (289^T, 290, and ATCC 35942) and *Aeromonas* and *Escherichia* strains used as references (see Tables 1 and 2) were grown in tryptone broth (1% [wt/vol] tryptone [Oxoid], 1% [wt/vol] NaCl; pH 7.2) at 25°C, and cells were harvested in late exponential phase. DNA was extracted and purified by the method of Marmur (14), and its purity was

assessed from the E_{260}/E_{280} and E_{230}/E_{260} ratios (12). The G+C content of the DNA was determined from the midpoint value (T_m) of the thermal denaturation profile (15) obtained with a Perkin-Elmer UV-Vis 551S spectrophotometer at 260 nm. This instrument was programmed for temperature increases of 1.0°C/min. The T_m was determined by a graphic method previously described (8), and the G+C content was calculated from this temperature by using the equation of Owen and Hill (18) in 0.1× standard SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The T_m of DNA from *Escherichia coli* NCTC 9001 was taken as a reference (see Table 1). DNA-DNA hybridization was conducted in triplicate by the competition procedure with the membrane method described by Johnson (12). DNAs from strains 289^T and ATCC 35942 were nick translated with [³H]dCTP (RPN 1601Y; Amersham International, Amersham, United Kingdom) and reacted with unlabeled competitor DNAs from the other *Aeromonas* strains at a reassociation temperature of 54°C. This hybridization temperature was selected according to the T_m of the DNA used as a labeled probe (12). The hybridization mixtures contained 30% formamide (Sigma). The ratio of the concentrations of competitor to labeled DNA was at least 150:1. After hybridization, the filters were washed in 2× SSC at the optimal renaturation temperature (54°C). The radioactivity

TABLE 1. T_m s and DNA base compositions of *A. allosaccharophila* strains and *E. coli* NCTC 9001

Bacterial strain	Mean T_m^a (°C)	G+C content (mol%) determined	
		In this study	Elsewhere ^b (reference)
<i>A. allosaccharophila</i> 289 ^T (= CECT 4199 ^T)	77.5 ± 0.4	56.9	59.5 (18)
290	77.0 ± 0.0	55.9	ND
ATCC 35942	77.7 ± 0.4	57.3	ND
<i>E. coli</i> NCTC 9001	74.6 ± 0.05 ^c	50.9	50.9 (20)

^a Values are averages of three or more separate determinations in 0.1× SSC buffer.

^b ND, not determined.

^c The T_m reported elsewhere is 74.6°C (20).

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TABLE 2. Levels of DNA-DNA homology between *A. allosaccharophila* strains and other *Aeromonas* species and strains

Source of unlabeled DNA	% Homology ^a with ³ H-labeled DNA from:	
	289 ^T (= CECT 4199 ^T)	ATCC 35942
<i>A. allosaccharophila</i>		
289 ^T (= CECT 4199 ^T)	100	94
290	100	94
ATCC 35942	70	100
<i>A. hydrophila</i> ATCC 7966 ^T (DNA group 1)	ND	0
<i>A. salmonicida</i> subsp. <i>salmonicida</i> (DNA group 3)		
NCIMB 1102 ^T	0	33
ATCC 14174	0	1
<i>A. caviae</i> ATCC 15468 ^T (DNA group 4)	0	41
<i>A. media</i> ATCC 33907 ^T (DNA group 5b)	35	0
<i>A. eucrenophila</i> NCIMB 74 ^T (DNA group 6)	22	0
<i>A. sobria</i> CIP 74.33 ^T (DNA group 7)	0	36
<i>A. veronii</i> biogroup <i>sobria</i> ATCC 9071 (DNA group 8/10)	0	40
<i>A. veronii</i> biogroup <i>veronii</i> ATCC 35624 ^T (DNA group 8/10)	0	0
<i>A. jandaei</i> ATCC 49568 ^T (DNA group 9)	0	13
<i>Aeromonas</i> sp. strain ATCC 35941 (DNA group 11)	0	11
<i>A. schubertii</i> ATCC 43700 ^T (DNA group 12)	0	0
<i>A. trota</i> ATCC 49657 ^T (DNA group 13)	0	0
<i>Aeromonas</i> sp. strain ATCC 43946 (enteric group 501)	0	0
<i>A. ichthiosmia</i> DSM 6393 ^T	0	0
<i>A. enteropelogenes</i> DSM 6394 ^T	ND	21

^a Each value is the mean from at least two independent experiments done in triplicate. ND, not determined.

bound to the filters was measured in a liquid scintillation counter (Beckman Instruments, Inc.), and the percentage of reassociation was calculated as described previously (12).

The G+C content of *A. allosaccharophila* 289^T (= CECT 4199^T) was 56.9 mol%, which is different from that previously described (17) (Table 1). This value, however, was very close to those determined for other *A. allosaccharophila* strains (Table 1). Thus, the G+C contents of the strains of *A. allosaccharophila* were between 55.9 and 57.3 mol% (Table 1). Interestingly, *A. allosaccharophila* presented a very characteristic G+C content compared with the values reported for the other mesophilic *Aeromonas* species, which are generally ≥ 58 mol% (3, 4, 9, 10, 21, 22–24).

The DNA homology values obtained between two representatives of *A. allosaccharophila* and all strains investigated are shown in Table 2. *A. allosaccharophila* 289^T (= CECT 4199^T) showed 70% or more DNA relatedness with the other *A. allosaccharophila* strains. Nevertheless, very low levels of homology (0 to 35%) were obtained between this strain and the reference strains studied, representing psychrophilic and mesophilic species and representatives of DNA homology groups of the genus *Aeromonas* (Table 2). Similar results (0 to 41% hybridization) were obtained when labeled DNA from *A. allosaccharophila* ATCC 35942 was used as a reference (Table 2). Therefore, our results clearly confirm that the strains 289^T (= CECT 4199^T), 290, and ATCC 35942 constitute a very homogeneous group genotypically, showing DNA reassociation levels ($\geq 70\%$) consistent with the currently accepted genospecies concept (19, 25). Likewise, these data support the hypothesis that the species *A. allosaccharophila* represents a new hybridization group of the genus *Aeromonas*. Tentatively, we propose to define this new hybridization group as group 14.

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