NOTE

Re-examining the 16S rDNA sequence of *Halomonas salina*Susanne Baumgarte,¹ E. R. B. Moore¹ and B. J. Tindall²Author for correspondence: B. J. Tindall. Tel: +49 531 2616 224. Fax: +49 531 2616 418.
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Previous reports of 16S rDNA sequencing of members of the family Halomonadaceae Franzmann *et al.* 1989 include two different sequences that were both attributed to the type strain of *Halomonas salina* (Valderrama *et al.* 1991) Dobson and Franzmann 1996 (basonym *Deleya salina* Valderrama *et al.* 1991). The two sequences are sufficiently different for them to belong to two different species within the genus *Halomonas*. In order to determine which of the two sequences corresponded to that of the type strain of *Halomonas salina*, the designated type strains of this species were obtained from the ATCC and the DSMZ. It was possible to show that only one of the previous sequences corresponded to the sequences obtained from DSMZ 5928^T and ATCC 49509^T.

Keywords: *Halomonas salina*, 16S rDNA sequence

Independent studies on the taxonomy of Gram-negative, aerobic, halotolerant rods led to the creation of two genera, *Halomonas* and *Deleya*. Members of the genus *Halomonas* were isolated initially from moderately to highly saline environments (Vreeland *et al.*, 1980), while members of the genus *Deleya* were isolated initially from marine (slightly saline) habitats (Baumann *et al.*, 1983). Examination of a larger number of isolates and the description of a larger number of species in the two genera showed that members of the genus *Deleya* could also be isolated from highly saline environments (Valderrama *et al.*, 1991). Studies on rRNA–DNA hybridization (De Vos *et al.*, 1989; Kersters, 1991) and 16S rRNA cataloguing (Franzmann *et al.*, 1988) provided an indication that members of these two genera shared a monophyletic origin and that members of the two genera could not necessarily be assigned to two distinct phyletic lineages corresponding to the genera *Deleya* and *Halomonas*. Chemical studies (Franzmann & Tindall, 1990) and 16S rDNA sequence analysis (Dobson *et al.*, 1993) confirmed that the members of the two genera did not form two exclusive lineages and that members of the two genera were intermixed with each other in such a fashion that it was not possible to define the genus *Deleya* without including species of the genus *Halomonas* or vice versa. Considering that it was not

possible to divide this group of organisms into two distinct genera, the proposal was made to include all members of this phyletic group in the genus *Halomonas* (Dobson & Franzmann, 1996), which includes not only members of the genus *Deleya*, but also organisms initially described as *Paracoccus halodenitrificans*, *Volcaniella eurihalina* and *Halovibrio variabilis* (Miller *et al.*, 1994; Mellado *et al.*, 1995).

During the course of independent studies on the 16S rDNA sequences of members of the genus *Halomonas*, a number of organisms were analysed in Braunschweig (Mellado *et al.*, 1995) and in Hobart (Dobson *et al.*, 1993; Dobson & Franzmann, 1996). Although the majority of the sequences obtained for the appropriate type strains of the different species were highly similar, indicating that the few differences observed may be due to sequencing errors or ambiguities in the primary sequence, the sequences of *Halomonas salina* (basonym *Deleya salina*) obtained in the two laboratories and deposited as X87217 and L42617 possessed approximately 5% similarity divergence. Such differences are not usually attributable to methodological problems, but are usually the result of problems associated with strain identity (see Clayton *et al.*, 1995). Considering that both laboratories reported the analysis of a strain of *Halomonas salina* derived from the type strain, F8–11, originally supplied from the same laboratory in Seville, Spain, the present work was undertaken to establish the 16S rDNA sequence of the type strain held in the DSMZ and the ATCC. Strains DSMZ 5928^T and ATCC 49509^T are both derived from strain

The EBI/EMBL accession numbers for the 16S rDNA sequences of *Halomonas salina* ATCC 49509^T and DSM 5928^T are AJ243447 and AJ243448, respectively.

F8-11 (Valderrama *et al.*, 1991), having been obtained in parallel from M. J. Valderrama, in Granada, Spain, and were used to clarify the discrepancy between results already reported for *Halomonas salina*.

Strains were grown on agar plates on the medium described by Valderrama *et al.* (1991) and checked for purity. Single colonies were taken directly for sequence analysis, suspended in 100 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and boiled for 5 min (Moore *et al.*, 1999). The lysate was centrifuged briefly and 0.5 µl of the supernatant was used for PCR in duplicate reactions for each strain. The 16S rRNA genes were amplified by PCR (Mullis & Faloona, 1987) using a forward primer hybridizing at (the complements of) positions 8–27 and a reverse primer hybridizing at positions 1525–1541 (*Escherichia coli* 16S rRNA gene sequence numbering). PCR and sequencing reactions were carried out under conditions described previously (Karlson *et al.*, 1993). The 16S rRNA sequencing primers have been described previously (Lane, 1991). The 16S rDNA sequences determined for *Halomonas salina* ATCC 49509^T (accession no. AJ243447) and DSM 5928^T (accession no. AJ243448) have been deposited in the EMBL nucleotide database.

The strains of *Halomonas salina* obtained from the DSMZ and the ATCC were originally deposited independently by M. J. Valderrama, so that the two culture collection strains should represent parallel cultures of the same strain. No evidence was found for contamination of either strain, nor did the two strains appear to be a mixture of two different organisms, based on colony morphology or cell shape and size. The 16S rDNA partial sequences of both cultures possessed 100% sequence similarity over a continuous stretch of 1493 bases (positions 28–1524, *E. coli* 16S rDNA sequence numbering). Although 16S rDNA sequence analysis is not usually sufficient to distinguish between different species when similarity values are higher than about 98%, no further work was undertaken to confirm whether the two strains were identical, since the published work on the 16S rDNA sequence clearly indicated that the two strains of *Halomonas salina* used in previous studies did not belong to the same species. The point of the study was to simply clarify the discrepancy observed in the previous studies (Mellado *et al.*, 1995; Dobson & Franzmann, 1996). Taking the strain history of DSMZ 5928^T and ATCC 49509^T into consideration, the 16S rDNA sequence data were taken to indicate that the same organism had been obtained from the two different culture collections. Alignment of the new, nearly complete sequences with those sequences already obtained in the two previous studies indicated that the sequences obtained in the present study are identical to that obtained by Dobson & Franzmann (1996) for *Halomonas salina*, when taking into consideration those positions which were determined unambiguously. We therefore conclude, based on the fact that the sequences for the strain used by Dobson &

Franzmann (1996) (accession no. L42617) as well as those obtained for DSMZ 5928^T (accession no. AJ243448) and ATCC 49509^T (accession no. AJ243447) are identical, that they are from the same organism. Evidently, all three strains may be considered to be derived from the same strain and to be clearly different from the organism sequenced by Mellado *et al.* (1995) (accession no. X87217). While the organism sequenced by Mellado *et al.* (1995) also bore the name *Deleya salina*, the 16S rDNA work carried out here and that of Dobson & Franzmann (1996) would indicate clearly that it is, in fact, a different and probably new species within the genus *Halomonas*. Unfortunately, the strain sequenced by Mellado *et al.* (1995) has not been maintained in Braunschweig, although it is obvious, in retrospect, that this strain would also have been interesting to study further, since it is clearly not identical to any currently described member of the genus *Halomonas* and the degree of 16S rDNA dissimilarity (about 5%) indicates that it represents a new species within this genus. Based on the present information, we can conclude that the sequence published under accession no. X87217 does not belong to the type strain of *Halomonas salina* and we have therefore corrected the entry made in the EMBL/DDJB/GenBank databases. It should be pointed out that, although 16S rDNA sequence data have made a valuable contribution to modern taxonomy, to such an extent that such sequencing has become a routine method in many laboratories, errors of the type described here do occur occasionally. While 16S rDNA sequence analysis has indicated that taxa such as *Micrococcus* and *Arthrobacter*, to name one example, comprise two overlapping polyphyletic morphological groups, bringing into question the established taxonomy, there are also examples, such as in the case of *Caulobacter subvibrioides*, where biochemical and chemical data contradicted the conclusions derived from 16S rDNA sequence data, the latter having been shown to be in error (Tindall, 1994; Abraham *et al.*, 1999). The present case illustrates further the need for care in the handling, maintenance and characterization of strains, the collection of data and the critical evaluation of data published previously.

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