Corynebacterium mooreparkense, a later heterotypic synonym of *Corynebacterium variabile*

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Strains of a Gram-positive bacterium were isolated from the Irish smear-ripened cheese Gubbeen, and assigned to a new species, *Corynebacterium mooreparkense*, in 2001. During a further study on the same cheese, no additional isolates from this species could be found. Instead, multiple isolates of its nearest phylogenetic neighbour, *Corynebacterium variabile*, were found. A first screening with rep-PCR and SDS-PAGE pointed to a similarity between *C. mooreparkense* and *C. variabile*. Following this peculiar result, attempts were made to collect all type strains deposited at different culture collections and all strains described by Brennan *et al.* [*Int J Syst Evol Microbiol* (2001) **51**, 843–852]. Subsequently, 16S rRNA gene sequencing and DNA–DNA hybridizations were performed. All *C. mooreparkense* strains had a 16S rRNA gene sequence similarity of at least 99.5% with *C. variabile* and the DNA–DNA relatedness was 95%. On the basis of these results, it is concluded that *C. mooreparkense* is a later heterotypic synonym of *C. variabile*.

Corynebacterium mooreparkense (LMG S-19265^T=LMG 19265^T=NCIMB 30131^T=DPC 5310^T) is a species whose strains were isolated from an Irish smear-ripened cheese (Gubbeen) and was described by Brennan *et al.* (2001). Ten isolates were assigned to the species by these authors: DPC 5305, DPC 5306, DPC 5307, DPC 5308, DPC 5309, DPC 5312, DPC 5313, DPC 5314, DPC 5315 and DPC 5310^T. The type strain was originally deposited in the BCCM/LMG Bacteria Collection as LMG S-19265^T and at the National Collection of Industrial and Marine Bacteria as NCIMB 30131^T.

During a recent research project, the surface microflora of Gubbeen was again thoroughly studied. A first screening method consisted of comparing the band patterns obtained with rep-PCR by using primer BOXA1R (Versalovic *et al.*, 1994). Twenty-five cheese-surface isolates showed a similar band pattern with the type strain of *C. mooreparkense*. However, all band patterns were also very similar to those of strains of *Corynebacterium variabile* (LMG 22560^T, LMG 22561 and LMG 22562). This peculiar result persuaded us to ask the author of the *C. mooreparkense* description to

send us all ten above-mentioned DPC isolates and we requested from NCIMB the strain deposited in their culture collection (NCIMB 30131^T). rep-PCR was repeated with all these strains. Fig. 1 shows that the rep-PCR band patterns obtained with primer BOXA1R of *C. mooreparkense* strains DPC 5305 to DPC 5310^T (LMG 19265^T) and NCIMB 30131^T are very similar to those of *C. variabile* strains and some recent Gubbeen isolates (numerical analysis was performed by using the Pearson coefficient and the UPGMA dendrogram type with the Bionumerics software; Applied Maths).

Representatives of these isolates were compared by SDS-PAGE of whole-cell proteins, a method that has been proven to differentiate on the species level (Pot *et al.*, 1994; Kersters & De Ley, 1975). Fig. 2 shows that there is no significant difference between both the type strains and other isolates of *C. mooreparkense* and *C. variabile*. Moreover, two recent representative isolates from Gubbeen cheese (LMG 22338 and LMG 22363) also show high similarity with *C. variabile* and *C. mooreparkense* (numerical analysis with Pearson coefficient and UPGMA dendrogram type using the GelCompar software; Applied Maths).

Next, we tried to collect all type strains of *C. mooreparkense* from the different institutes involved in the original description or available in other culture collections. The University of Newcastle, where the 16S rRNA gene

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of LMG 19265^T and LMG 22560^T are AJ783438 and AJ767054, respectively.

Fig. 1. rep-PCR patterns obtained with primer BOXA1R of the ten DPC strains described by Brennan *et al.* (2001), NCIMB 30131^T, representative cheese isolates and *C. variabile* strains [cheese isolates with *, strains isolated recently (2003) from Gubbeen cheese].

sequencing was performed, and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), where the DNA–DNA hybridization was performed, were contacted. However, these research groups disposed of the strain after analysis. The ten strains from the DPC collection and NCIMB 30131^T were therefore used for partial (~570 bp) 16S rRNA gene sequences. All strains showed a similarity of at least 99.5 % among each other (data not shown) and with the newly determined complete sequence of *C. mooreparkense* LMG 19265^T (GenBank/EMBL/DDBJ accession no. AJ783438), the type strain of *C. variabile* LMG 22560^T sequenced in our laboratory (AJ767054) and the deposited sequence of *C. variabile* NCDO 2097^T (X53185). However, a similarity of only 96.6 % was obtained with the original sequence of *C. mooreparkense* LMG 19265^T (AF267148) of Brennan *et al.* (2001) (Fig. 3). This could be explained by mistakes during the sequencing procedure, which resulted in many inserts that inexplicably can be found in the 16S rRNA gene sequences of DPC 5305, DPC

5307, DPC 5315 and DPC 5310^T (GenBank/EMBL/DDBJ accession nos AF267149, AF267151, AF267150 and AF267148, respectively) but cannot be found in any other strain of *C. mooreparkense* when re-sequenced in our laboratory.

In a following step, DNA–DNA hybridizations between *C. variabile* LMG 22560^T and *C. mooreparkense* LMG 19265^T were performed. The G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989) using the further specifications given by Logan *et al.* (2000). DNA–DNA hybridization was performed by using a modification of the microplate method of Ezaki *et al.* (1989), as described by Willems *et al.* (2001). A hybridization temperature of 47 °C (calculated with correction for the presence of 50 % formamide) was used. A relatedness of 95 % between LMG 22560^T and LMG 19265^T was obtained (reciprocal values of 91.4 and 98.9 %), thus assigning the two strains to a single species, according to the recommendations for

Fig. 2. SDS-PAGE band patterns and corresponding dendrogram of representative isolates of *C. mooreparkense* and *C. variabile* [cheese isolates with *, strains isolated recently (2003) from Gubbeen cheese].

Fig. 3. Phylogenetic relationships of *C. variabile*, *C. mooreparkense* and their closest neighbours based on neighbour-joining analysis of the 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. *C.*, *Corynebacterium*; *R.*, *Rhodococcus*.

species designation (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002). This value is in contradiction with the value obtained by Brennan *et al.* (2001), who state that the level of DNA re-association is 26 %.

We conclude that the strains described as *C. mooreparkense* match the genomic characteristics of *C. variabile* and that *C. mooreparkense* must therefore be seen as a later heterotypic synonym of *C. variabile*.

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