Gram-positive anaerobic cocci

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Chapter 5

The mistaken identity of

Peptoniphilus asaccharolyticus

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Data letter

Peptoniphilus (*Pn.*) *asaccharolyticus* is a commonly isolated gram-positive anaerobic coccus (GPAC) [7]. However, the type strain ATCC14963 is not representative for the species. Huss et al. [4] described that the DNA-DNA homology between the type strain and clinical isolates was < 25%.

Because of this finding new species were described [6], among them *Pn. harei*. *Pn. harei* has the same biochemical features as *Pn. asaccharolyticus* and can only be differentiated from *Pn. asaccharolyticus* by its irregular colony and cell morphology [5]. The clinical relevance of *P. harei* was unknown. However, in studies of clinical isolates using molecular techniques for identification a remarkable number of *Pn. harei* was found. Song et al. [9] identified 25.3 % of all GPAC as *Pn. harei*. In another study [10] 17.0 % was identified as *Pn. harei* by fluorescent *in situ* hybridisation. In both studies, no *Pn. asaccharolyticus* was encountered. To substantiate the genotypic identity of *Pn. asaccharolyticus* reference strains were needed. To this end, a number of type strains were re-identified using 16S rRNA gene sequencing. These were *Pn. asaccharolyticus* strains from the Culture Collection of the University of Göteburg (Sweden) CCUG42643, CCUG43862, CCUG44165, CCUG47015, and CCUG48151. DNA was isolated and amplified as described [1, 2]. Sequences were aligned and a filter was set at *Escherichia coli* positions 257 and 1436. Sequence similarities with closest relatives and *Pn. asaccharolyticus* were calculated using the DNA distance matrix in BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

For each strain the closest relative was *Pn. harei*, with sequence similarities between 99.0 and 99.4 % (Table 1). The sequence similarity with *Pn. asaccharolyticus* was between 89.2 and 89.6 %. The original identification of these strains was based on their biochemical features. Since, *Pn. harei* and *Pn. asaccharolyticus* share the same biochemical features it is clear that these strains were misidentified in the past.

Song et al. [8] developed a flow chart for the phenotypical identification of GPAC. It is mentioned that the alkaline phosphatase test might be useful to differentiate the species from each other. The sequence similarity of *Pn. asaccharolyticus* strain ATCC29743 with *Pn. harei* was 99.6 % (Table 1), indicating that it is not *Pn. asaccharolyticus*. However, in the study of Song et al. [8] this strain was assumed to be *Pn. asaccharolyticus*. This confirms that *Pn. harei* and *Pn. asaccharolyticus* cannot be differentiated from each other phenotypically. Holdeman-Moore et al. [3] commented already in 1986 that one should be cautious in reporting on isolation and incidence of *Pn. asaccharolyticus*. In our opinion this caution still stands. The fact that the type strain of *Pn. asaccharolyticus* ATCC14963 is atypical for clinical isolates might be due to the true identity of the

clinical isolates used for comparison. This can explain the low DNA-DNA homology [4] between the type strain and clinical isolates.

We are convinced that the incidence of *Pn. asaccharolyticus* in clinical material is highly overestimated. The clinical importance of *Pn. harei* in the pathogenesis of anaerobic infections still has to be defined.

The 16S rRNA sequences of strains of CCUG42643, CCUG43862, CCUG44165, CCUG47015, and CCUG48151 have been deposited in Genbank under accession numbers HQ326629, HQ326630, HQ326631, HQ326632, and HQ326633, respectively.

Table 1. Sequence similarities of the 16S rRNA genes between the type strains of *Pn. harei* and *Pn. asaccharolyticus,* and several strains which were originally identified as *Pn. asaccharolyticus*.

Strain	% similarity	
	Pn. harei ATCC BAA-601^T	*Pn. asaccharolyticus* ATCC 14963^T
ATCC 29743 (DQ986463)	99.4	89.5
CCUG 42643 (HQ326629)	99.1	89.2
CCUG 43862 (HQ326630)	99.0	89.4
CCUG 44165 (HQ326631)	99.3	89.4
CCUG 47015 (HQ326632)	99.2	89.3
CCUG 48151 (HQ326633)	99.3	89.3
Pn. harei ATCC BAA-601^T	100	89.6

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