

Lineages with broad dairy biotope ranges and phenotypic variability in *Propionibacterium freudenreichii* revealed by multilocus sequence typing.

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Abstract:

Propionibacterium freudenreichii is used as a ripening culture in Swiss cheese manufacture. Moreover, *P. freudenreichii* is studied for its potential probiotic capacities such as the modulation of intestinal inflammation, of microbiota in gut, of immune system and of cancer development. In this study, the molecular diversity and the population structure, specifically the relationships between *P. freudenreichii* strains, were investigated using multilocus sequence typing (MLST). Internal fragments of seven genes were amplified by PCR and sequenced for 113 strains of different subspecies and dairy origins. The MLST scheme resolved 46 sequence types (STs) with occurrence frequencies ranging from 1 to 11. Our results indicate a low level of nucleotide polymorphism in the core genome of the species: single nucleotide polymorphisms accounted for only 2.28% of the concatenated sequence, and the average polymorphism rate in pairwise comparison was 0.46%. The results also show that recombination played a significant role in the distribution of this polymorphism among isolates. Remarkably, the lineages defined by the STs exhibited little if any dairy biotope specialization. Furthermore, subspecies identification of the strains, based on their aptitude to use lactose and nitrate, revealed considerable phenotypic heterogeneity of the lineages. Namely, some strains of different origins and of different subspecies phenotypes displayed the same STs whereas strains of the same origin or subspecies phenotypes were disseminated over the phylogenetic tree. This study brings new insights into the population structure and the mode of evolution of *P. freudenreichii*. It also constitutes the first development of MLST, an effective molecular typing tool for future screening of strain diversity in larger collections.

Key words: multilocus sequence typing, polymorphism, *Propionibacterium freudenreichii*, subspecies