Available online at www.sciencedirect.com
SCIENCE DIRECT
Systematic and Applied Microbiology 29 (2006) 463–469
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Evaluation of *rec*A gene as a phylogenetic marker in the classification of dairy propionibacteria ☆

Franca Rossi, Franco Dellaglio, Sandra Torriani*

Dipartimento Scientifico e Tecnologico, Università degli Studi di Verona, Strada Le Grazie 15, 37134, Verona, Italy

Received 1 December 2005

Abstract

The aim of this study was to investigate the validity of *rec*A gene as a molecular marker for the reliable discrimination and classification of dairy propionibacteria and the closely related species. Regions of the *rec*A gene, varying in size between 613 and 677 nucleotides, were sequenced for *Propionibacterium acidipropionici*, *P. cyclohexanicum*, *P. freudenreichii*, *P. jensenii*, *P. microaerophilum* and *P. thoenii* using degenerate consensus primers constructed by aligning *rec*A sequences of some actinobacteria. The 16S rRNA encoding genes for the type and reference strains of the species *P. acidipropionici*, *P. jensenii* and *P. thoenii* were also sequenced to remove ambiguous positions present in the current database reports, such to improve the classification scheme of reference. As found for other bacterial species, *rec*A sequences permitted a better distinction among the dairy propionibacteria considered than 16S rRNA gene. However, the topology of phylogenetic trees constructed on the *rec*A gene regions sequenced and their putative translations appeared rather different and less statistically valid than the 16S rRNA gene tree. In addition, the possibility of designing PCR-based identification and detection tests on the new *rec*A sequences was demonstrated by assessing specific amplification protocols for *P. cyclohexanicum* and *P. microaerophilum*.

Keywords: Dairy propionibacteria; Phylogenetic markers; 16S rRNA; *Rec*A gene; Identification; Species-specific PCR; *P. cyclohexanicum*; *P. microaerophilum*

Introduction

Dairy, or classical, propionibacteria are an important group of microorganisms in the cheesemaking technology. They colonize raw milk, or are added as starter cultures, and are essential to achieve sensorial peculiarities in Swiss-type cheeses and other hard cheeses. Beyond *Propionibacterium acidipropionici*, *P. freudenreichii*, *P. jensenii* and *P. thoeni*, [2,3], new species related to classical propionibacteria were more recently proposed, i.e. *P. cyclohexanicum* [12], isolated from spoiled orange juice and closely related to *P. freudenreichii*, and *P. microaerophilum*, isolated from fermented olives and

☆ *Note*: Nucleotide sequence data reported for 16S rRNA and partial *rec*A genes are available in the DDBJ/EMBL/GenBank databases under the accession numbers, respectively: AJ704569 and AJ704573 (*P. acidipropionici* NCFB 563^T), AJ704570 and AJ704575 (*P. acidipropionici* NCFB 570), AJ704571 and AJ704576 (*P. jensenii* DSM 20535^T), AJ937773 and AJ704577 (*P. jensenii* NCFB 571), AJ704572 and AJ715965 (*P. thoenii* NCFB 568^T). Accession number for the *rec*A gene of *P. freudenreichii* NCFB 853^T is AJ704574. Accession numbers for partial *rec*A genes of *P. cyclohexanicum* NCIMB 13575^T and *P. microaerophilum* DSM 13435^T are AJ715966 and AJ715964, respectively.

*Corresponding author. Tel.: +39 045 8027921; fax: +39 045 8027051.
E-mail address: sandra.torriani@univr.it (S. Torriani).

0723-2020/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2006.01.001

proximate to *P. acidipropionici* [11]. The taxonomy of propionibacteria currently relies on classical genetic methods such as DNA–DNA hybridization and 16S rRNA gene sequence comparison. Some results on the phylogeny and identification according to the sequences of 16S–23S [4] and 23S–5S [19] ribosomal spacer regions have also been presented. Differently than for other bacterial groups, the sequence analysis of protein coding genes suitable as molecular phylogenetic markers has not yet been considered. However, for microorganisms used in food technology like propionibacteria, the conceiving of gene sequencing tests more capable of distinguishing ecological and phenotypic variants is particularly worth of attention.

Comparative analysis of 16S rRNA gene sequence is in excellent concordance with DNA–DNA hybridization for establishing bacterial classification schemes [16] and has been extensively used in designing PCR tests for identification of bacteria. Therefore it remains the basis for evaluating the plausibility of hierarchical orders established by comparison of protein-coding genes that show greater evolutionary rates [9]. However, it presents pitfalls like no discrimination of genetically closely related, but phenotypically distinct, *taxa* and bias in the estimation of the degrees of relatedness due to its poor representation of different G + C genome contents. The current 16S rRNA gene-based classification of dairy propionibacteria avails itself of database reports with several ambiguous nucleotide positions for the species *P. acidipropionici*, *P. jensenii* and *P. thoenii*. We experienced that the recent introduction in the public databases of a more complete 16S rRNA gene sequence for strain *P. acidipropionici* DH4 [14] might render problematic the identification of new classical propionibacteria isolates. Particularly, we observed that the type strains of the species *P. jensenii* and *P. thoeni* matched the sequence of *P. acidipropionici* DH4 better than the old data available for the respective species, leading to their misidentification as *P. acidipropionici*. This underlined the need to re-sequence the 16S rRNA gene for the species *P. acidipropionici*, *P. jensenii* and *P. thoeni*. Furthermore the construction of a more reliable SSU rRNA classification scheme, based on more complete sequence data, is required to investigate the validity of alternative molecular markers, such as *rec*A, in the definition of bacterial phylogenesis.

RecA protein has been considered a good candidate for inferring classification schemes because of its conserved sequence and function in bacteria; in fact it plays a central role in homologous recombination, DNA repair and regulation of the SOS response. In the studies regarding its possible application as a phylogenetic marker it maintained an overall consistency with 16S rRNA-based phylogeny and permitted a better resolution of taxonomic groups with high 16S rRNA similarity [1,6,13,18,20]. In some instances, *rec*A gene sequence comparison allowed the discrimination of *taxa* that could not be separated by 16S rRNA sequence analysis [1,13,18].

In this study a classification of dairy propionibacteria based on the *rec*A gene sequences was carried out to evaluate its eligibility for deciphering their phylogeny and a better separation of the species of this group. A region of the *rec*A gene was sequenced for representative strains of *P. acidipropionici*, *P. cyclohexanicum*, *P. freudenreichii*, *P. jensenii*, *P. microaerophilum* and *P. thoenii* by using degenerated consensus primers designed by aligning *rec*A sequences of some actinobacteria. Phylogenetic trees based on both nucleotide and derived amino acid sequences, were compared with the 16S rRNA gene phylogeny constructed with the renewed sequence data for *P. acidipropionici*, *P. jensenii* and *P. thoenii*. Finally, the *rec*A sequences were used to develop specific PCR tests for the identification of *P. cyclohexanicum* and *P. microaerophilum*, not yet available for these species.

Materials and methods

Bacterial strains and growth conditions

The dairy propionibacteria strains used in this study for DNA preparations are from public collections and are reported in the figures. All strains were grown in a medium formulated as follows: 1% (w/v) sodium lactate, 1% (w/v) tryptone, 1% (w/v) yeast extract, 0.25% (w/v) K_2HPO_4, 0.12% (w/v) KH_2PO_4, pH 7.0. Incubation was done at 30 °C, in anaerobiosis, for at least 48 h, until reaching the stationary growth phase.

Genomic DNA extraction

For genomic DNA preparation, cells were harvested from 1 ml culture and lysed with 10 mg ml^{-1} lysozyme in 1ml Tris/HCL_1 pH 8.0 for 30 min at 37 °C; then SDS was added to the final concentration of 0.5%. The nucleic acid suspension was treated with 100 μg ml^{-1} RNAse for 30 min at 37 °C and subsequently with 20 μg ml^{-1} proteinase K for additional 30 min at 60 °C. Sodium perchlorate was added to 1 mol l^{-1} final concentration and DNA was purified by one step chloroform extraction. Finally, DNA was precipitated with absolute ethanol, washed with 70% (v/v) ethanol and resuspended in 50 μl sterile bi-distilled water.

Primer selection and PCR conditions

Primers used to amplify and sequence regions of *rec*A and 16S rRNA genes for each species and their annealing positions are reported in Tables 1 and 2, respectively. The following *rec*A gene sequences were