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Review

New insights into physiology and metabolism of *Propionibacterium freudenreichii*

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ABSTRACT

Dairy propionibacteria are Actinobacteria, mainly isolated from dairy environments. *Propionibacterium freudenreichii* has been used for a long time as a ripening culture in Swiss-type cheese manufacture, and is more and more considered for its potent probiotic effects. This review summarises the knowledge on the main *P. freudenreichii* pathways and the main features explaining its hardiness, and focuses on recent advances concerning its applications as a cheese ripening agent and as a probiotic for human health. Propionibacteria have a peculiar metabolism, characterised by the formation of propionic acid as main fermentation end-product. They have few nutritional requirements and are able to use a variety of carbon substrates. From the sequence of *P. freudenreichii* CIRM-BIA1^T genome, many pathways were reconstituted, including the Wood–Werkman cycle, enzymes of the respiratory chain, synthesis pathways for all amino acids and many vitamins including vitamin B_{12}. *P. freudenreichii* displays features allowing its long-term survival. It accumulates inorganic polyphosphate (polyP) as energy reserve, carbon storage compounds (glycogen), and compatible solutes such as trehalose. In cheese, *P. freudenreichii* plays an essential role in the production of a variety of flavour compounds, including not only propionic acid, but also free fatty acids released via lipolysis of milk glycerides and methyl-butanoic acids resulting from amino acid degradation. *P. freudenreichii* can exert health-promoting activities, such as a bifidogenic effect in the human gut and promising immunomodulatory effects. Many *P. freudenreichii* properties involved in adaptation, cheese ripening, bio-preservation and probiotic effects are highly strain-dependent. The elucidation of the molecular mechanisms involved is now facilitated by the availability of genome sequence and molecular tools. It will help in the selection of the most appropriate strain for each application.

Contents

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1. Introduction

Propionibacteria are Gram positive, high G + C%, mesophilic, aerotolerant, pleomorphic rods. They have a peculiar metabolism leading to the formation of propionic acid as main end-product of fermentation. They display low nutritional requirements and are characterised by their long-term survival in different environments.

P. freudenreichii was first described more than one century ago in Swiss Emmental cheese by Orla Jensen and von Freudenreich (von Freudenreich and Orla-Jensen, 1906), who showed the relationship between the presence of these bacteria producing propionic acid and the formation of the characteristic round holes (eyes) in cheese.

P. freudenreichii is widely used as a ripening culture in the manufacture of Swiss-type cheeses (Dorner, 1939; Langsrud and Reinbold, 1973a; Steffen et al., 1993). It is also known for its production of vitamin B_{12} and propionic acid, and has properties as protective cultures in food and feed (Thierry et al., 2011). Moreover *P. freudenreichii* is also more and more studied for its probiotic properties (Cousin et al., 2010).

This review summarises the knowledge on the main metabolic pathways of *P. freudenreichii* and the main characteristics explaining its hardiness, and focuses on recent advances concerning its applications as a cheese ripening agent and as a probiotic for human health.

2. General features of propionibacteria

2.1. Propionibacteria taxonomy

Propionibacteria belong to the Actinobacteria class, comprising high G + C content Gram-positive bacteria (Stackebrandt et al., 1997). Actinobacteria exhibit a wide range of morphologies, lifestyles, physiological and metabolic properties and colonise various niches including soil (Ventura et al., 2007) The *Propionibacterium* genus currently comprises 12 species (http://www.bacterio.cict.fr). Two distinct groups are distinguished on the basis on their natural biotopes: "dairy" (or "classical") and "cutaneous" propionibacteria (Cummins and Johnson, 1986). Dairy propionibacteria have been traditionally isolated from milk and dairy products. Four typical dairy species were early described: *P. freudenreichii*, *P. acidipropionici*, *P. jensenii* and *P. thoenii*. Cutaneous propionibacteria are commensal of mammals including humans, the most studied species within this group being *P. acnes,* involved in acne and in post surgery infections. Four propionibacteria species, isolated from other biotopes than dairy products and human skin, were more recently described: *P. cyclohexanicum* isolated from spoiled orange juice (Kusano et al., 1997), *P. microaerophilum* from olive mill wastewater (Koussemon et al., 2001), *P. australiense* from granulomatous bovine lesions (Bernard et al., 2002), and *P. acidifaciens*, isolated from human mouth (Downes and Wade, 2009). The analysis of 16S rRNA gene sequences showed that *P. cyclohexanicum*, *P. acidifaciens* and *P. australiense* are phylogenetically related to *P. freudenreichii*, whereas *P. microaerophilum* is related to *P. acidipropionici* (Downes and Wade, 2009).

2.2. Genetic diversity of P. freudenreichii

The genome size of *P. freudenreichii* ranges around 2.6 Mb and its G + C content is 67%. The first genome of a *P. freudenreichii* strain (CIRM-BIA1^T) has recently been sequenced (Falentin et al., 2010a). Furthermore, molecular tools have newly been developed to over pass *P. freudenreichii*'s poor efficiency of transformation (from 10^3 to 10^8 colony-forming units (cfu)/μg of DNA), thus allowing the exploration of the role of specific genes of interest (Thierry et al., in 2011; Van Luijk et al., 2002).

Data on genomic biodiversity within *P. freudenreichii* species are scarce. Fingerprinting methods such as Pulsed-Field Gel Electrophoresis and Randomly Amplified Polymorphic DNA-PCR are available to characterise *P. freudenreichii* at the strain level (Meile et al., 2008). Multi-locus sequence typing (MLST) based on the sequence analysis of internal fragments of 7 genes has recently been developed for *P. freudenreichii*. It was applied to 113 strains of different phenotypes and origins (Dalmasso et al., 2011). MLST resolved 46 sequence types grouping each 1 to 11 strains. This study showed that the *P. freudenreichii* core genome possesses a low level of nucleotide polymorphism and that recombination played a significant role in the distribution of this polymorphism among isolates (Dalmasso et al., 2011).

P. freudenreichii was in the past divided into two subspecies on the basis of lactose fermentation and nitrate reductase activity. *P. freudenreichii* subsp. *freudenreichii* is unable to ferment lactose and shows nitrate reductase activity whereas *P. freudenreichii* subsp. *shermanii* exhibits the opposite properties (Cummins and Johnson, 1986). However, the existence of *P. freudenreichii* strains harbouring the two other possible phenotypes (lactose and nitrate reductase positive, and lactose and nitrate reductase negative) has also been mentioned (Dalmasso et al., 2011; de Carvalho et al., 1994; Moore and Holdeman, 1986; Vorobjeva, 1999). In the same way, annotation of the complete *P. freudenreichii* CIRM-BIA1^T genome revealed that the *lacZ* gene responsible for betagalactosidase activity is harboured by a transposon-like mobile element and that the locus responsible for nitrate reduction activity harbours some pseudogenes (Falentin et al., 2010a). Moreover, the phylogenetic analysis of *P. freudenreichii* population using MLST did not fit with the distinction of subspecies (Dalmasso et al., 2011). All these results suggest that there seems to be no justification for separation at the subspecies level according to these two phenotypic criteria.

2.3. Propionibacteria ecology

Strains of dairy origin constitute the main part (80 to 90%) of the *P. freudenreichii* strains available in the international collections (for example, in the Belgian Co-ordinated Collections of Micro-organisms (BCCM™)/LMG collection and in the CIRM-BIA (Centre International de Ressources Microbiennes — Bactéries d'Intérêt Alimentaire, INRA, Rennes, France), and in other large laboratory collections (Fessler, 1997). However, a few *P. freudenreichii* strains have also been isolated from other biotopes, like hay and straw. Its known habitats are related to cattle environment but would require further investigations, since no systematic search has been made for propionibacteria in habitats distinct from dairy and cattle environments.

In most cheeses, *P. freudenreichii* and other dairy propionibacteria are present at low population density. In Emmental and similar cheeses, however, *P. freudenreichii* reaches a high population density, with counts over 10^9 cfu/g of cheese during the whole ripening time. The high thermotolerance of *P. freudenreichii,* compared to the other dairy species, would be responsible for the prevalence of this species