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# Identification of a glucan synthase gene potentially coding for exopolysaccharide production by *Propionibacterium freudenreichii* subsp. *shermanii*

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## Context

The dairy propionibacteria are generally considered as food-grade. Some strains of *Propionibacterium freudenreichii* subsp. *shermanii* (PFS) are also considered as potential probiotic organisms. In lactic acid bacteria, exopolysaccharides (EPS) have been shown to have a positive effect on the probiotic activity of certain strains. For the propionibacteria, EPS production remains little documented.

The aim of this work was to discover the genes responsible for EPS production by PFS.

## Methods & results

### Analysis of the genome of PFS CIP 103027<sup>T</sup>

According to the complete genome of PFS CIP 103027<sup>T</sup> (=TL34), a gene, chromosomally located, potentially coding for EPS biosynthesis was identified (named *pps*). The gene *pps* is composed of 1536 nt. The protein product Pps (511 aa) best aligns with Tts, a polysaccharide synthase gene of *St. pneumoniae* type 37. Tts catalyzes the synthese of a  $\beta$ -D-glucan capsule.

### Detection of *pps* in different strains

Specific primers were design of to detect *pps* among a number of strains and species (Fig 1). All 12 strains of PFS tested presented a “ropy” phenotype.

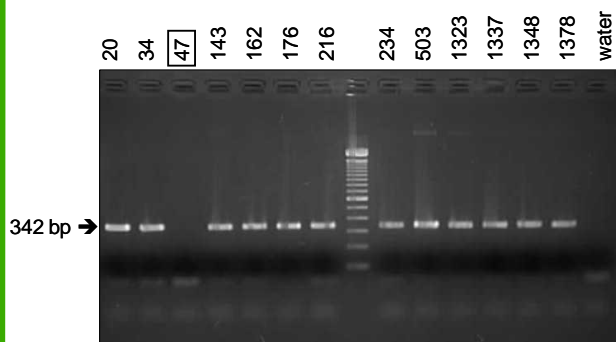


Fig 1: Agarose gel electrophoresis of PCR amplifcons of *pps* using total genomic DNA as template. All strains are PFS, excepted TL47 which is *P. acidipropionicii*.

⇒ The gene *pps* is detected in all the PFS strains tested.

⇒ Not detected in *P. acidipropionicii*, where the gene is not present or too divergent in sequence for the primers.

### Analysis of *pps* gene expression

Total RNA of strains was extracted for TL34, TL1337 & TL1378 (exponential growth phase). The presence of RNA was checked by one step RT-PCR (Fig 2).

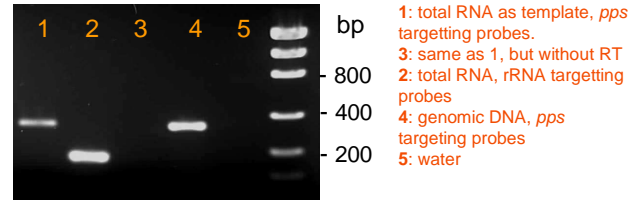


Fig 2: Agarose gel after one step-RT PCR amplification of total RNA of TL 34. (same results obtained for the strains TL1337 and TL1378)

⇒ The gene *pps* is expressed in the 3 strains tested.

### Detection of capsular EPS by immunological analysis

Agglutination tests were performed on PFS cultures with *St. pneumoniae* type-37 specific antiserum. This antiserum is specific for  $\beta$ -D-glucan.

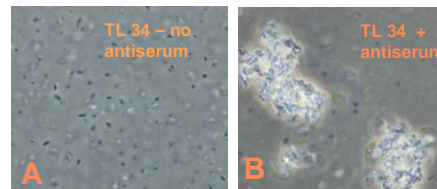


Fig 3: Immunological detection of a  $\beta$ -D-glucan capsule in strain TL34. The assays were performed with culture at exponential and stationary growth phases.

When the cells agglutinated in presence of antiserum, this indicates the presence of a  $\beta$ -D-glucan type capsule. Agglutination was positive for 7 out of 12 strains. TL34 was positive, whereas TL 1337 & TL1378 were negative. In the case of negative strains, the “ropy” phenotype observed is not due to the presence of a  $\beta$ -D-glucan capsule.

## Conclusions & perspectives

- ◆ First identification of an EPS encoding gene in PFS, present in all the strains tested.
- ◆ The results strongly suggest that *pps* encodes for a glucan synthase.
- ◆ A  $\beta$ -D-glucan type capsule has been detected for 7 out of 12 strains of PFS, but *pps* is present in the 12 strains:
  - Presence of another EPS-encoding gene in the genome ?
  - The *pps* gene may not be functional in all the strains ?
- ◆ Is the capsule related to the probiotic status of the *P. freudenreichii* strains ?

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