



**HAL**  
open science

## Combining genomic, proteomic, NMR and physiological study to understand salt stress response in *Propionibacterium freudenreichii*.

Guillaume Meurice, Pauline Leverrier, Annette Rouault, Marie-Bernadette Maillard, Anne Thierry, Christian Corre, Daniel Jacob, Diliانا Dimova, Catherine Deborde, Gwénaél Jan

### ► To cite this version:

Guillaume Meurice, Pauline Leverrier, Annette Rouault, Marie-Bernadette Maillard, Anne Thierry, et al.. Combining genomic, proteomic, NMR and physiological study to understand salt stress response in *Propionibacterium freudenreichii*. Congrès RMN Biologique et les Enjeux du Vivant., Mar 2003, Clermont-Ferrand, France. hal-03822231

**HAL Id: hal-03822231**

**<https://hal.inrae.fr/hal-03822231>**

Submitted on 20 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Combining genomic, proteomic, NMR and physiological study to understand salt stress response in *Propionibacterium freudenreichii*.



Guillaume Meurice<sup>a†</sup>, Pauline Leverrier<sup>a,b†</sup>, Annette Rouault<sup>a</sup>, Marie-Bernadette Maillard<sup>a</sup>, Anne Thierry<sup>a</sup>, Christian Corre<sup>a</sup>, Daniel Jacob<sup>a</sup>, Diliانا Dimova<sup>a</sup>, Catherine Deborde<sup>a</sup> and Gwénaél Jan<sup>a</sup> (Project Leader).

<sup>a</sup> INRA-LRTL, 65 rue de Saint-Brieuc, 35042 Rennes cedex. Phone: 02 23 48 53 45. Fax: 02 23 48 53 50, Mail: [cdeborde@labtechno.roazhon.inra.fr](mailto:cdeborde@labtechno.roazhon.inra.fr)  
<sup>b</sup> Standa Industrie, 68 rue Robert Kaskoreff, 14050 Caen cedex 4. † These authors contributed equally to this work

## What and Why dairy propionibacteria ?

### General characteristics

- High GC Gram-positive bacteria
- Peculiar carbon metabolism
- Particularly stress tolerant
- Anaerobic to aerotolerant
- Propionic acid, acetic acid and CO<sub>2</sub> production



© F. Michel & A. Rouault. Inra, France



© Sigf. France

255 000 t in France, 1<sup>st</sup> world producer of emmental, 600 000 t in Europe, € 2 billion/year.

### Industrial significance

- High economical impact:
- Ripening flora of the third most produced cheese
  - Bio-preservative of food and non-food products
  - Used in biotechnology (production of **vitamin B12**)

### Health significance

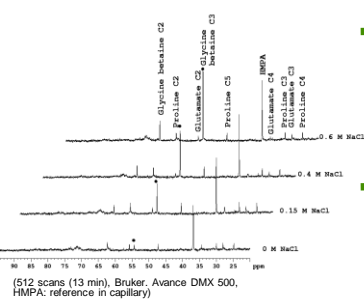
- Generally Recognized As Safe (GRAS status)
- Animal growth-promoting probiotic additive
- **Human probiotic**
- Apoptosis induction of colon cancer cells

(Jan G et al., 2002, Cell Death Differ. 9:179-188)

**Question:** Whatever the processes, vitamin B12 production or Swiss cheese manufacture propionibacteria are subjected to many technological stresses and especially the osmotic one.  
**How propionibacteria survive to osmotic stress?**

## Our multi-disciplinary approach

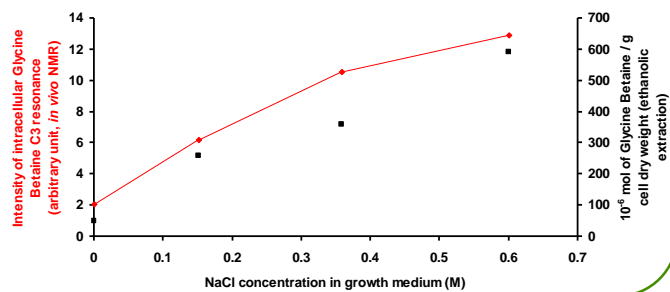
### In vivo <sup>13</sup>C NMR data



→ In vivo natural abundance <sup>13</sup>C NMR spectra of suspension of *P. freudenreichii* grown on Yeast Extract Lactate + NaCl medium

→ Major osmolytes => Glycine Betaine (GB), trehalose, proline and glutamate.

### Accumulation of Glycine Betaine by *P. freudenreichii*



### Genomic data and in silico analysis

#### Genomic data (Sept. 2002)

*P. freudenreichii* ssp. *shermanii*  
 Strain: CIP 103027 (ATCC9614)  
 Shotgun sequencing strategy

- genome coverage ~ 92%
- draft of 513 contigs
- high GC-content ~ 67%
- candidate genes ~ 2500

#### Bio-informatic tools

Development of a web interface, ContigBrowser<sup>®</sup>, for efficient genomic annotation and in silico metabolic reconstruction.

Contig	Contig	Size	Contig	Size	GC	Observation
1	135	for	1447	654 (792 %)	22042.60	

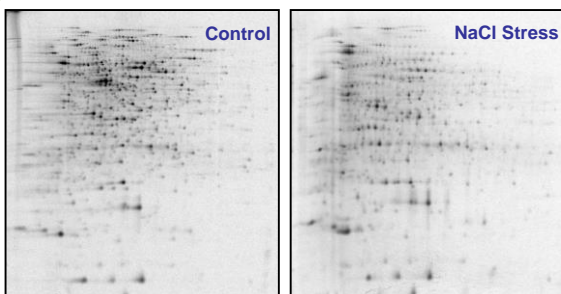
  

ACC	Nom de l'homologue	BLAST	Score	E-value	%Ident
YNM4	PL_126156 prolyle personee binding protein; coagulation; -Drosophila melanogaster	pf_cer7f.04	233.00	65.40	64.10
QPK4B	Q02682 (OPU4C) PRO5C	pf_cer7f.04	223.00	65.10	64.10
ACM4B	PL1ACT0613 prolyle ABC transport protein; binding protein; CT1571 (sequenced) - Salmonella enteritidis	pf_cer7f.04	164.00	39.40	35.90
QPK4C	Q02682 (OPU4C) Prolyle personee binding protein; coagulation; -Drosophila melanogaster	pf_cer7f.04	164.00	39.30	35.90
QPK4D	Q02682 (OPU4C) Prolyle personee binding protein; coagulation; -Drosophila melanogaster	pf_cer7f.04	164.00	39.30	35.90

All genes encoding proteins involved in GB and proline uptake systems and biosynthesis have been systematically searched in the actual *P. freudenreichii* genome draft:

- Evidence for transporters similar to *B. subtilis* ones:
  - OpuC and OpuE
- Absence of OpuD and OpuA transporters
- De novo biosynthesis of GB seems impossible
- Biosynthesis of GB from precursors such as choline and GB aldehyde seems possible:
  - Alcohol dehydrogenase EC 1.1.1.1 (*adh1*; AJ508921)
  - Betaine-aldehyde dehydrogenase EC 1.2.1.8 (*dha1*; AJ508920)
- GB biosynthetic pathway is similar to the *B. subtilis* one.

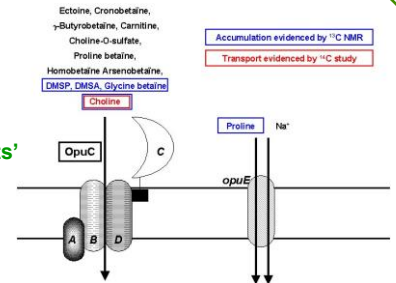
### Proteomic data



→ Reference 2D database - available  
 Salt stress-induced variations - detected  
 Protein identification: high-throughput mass spectrometry and de novo sequencing (MS/MS)

### Conclusions:

#### In silico model of osmoprotectants' transport in *P. freudenreichii*



- Physiological observations of GB and choline accumulation in *P. freudenreichii* are supported by genomic data.
- In silico evidence for a multicomponent transport system of choline, GB and other osmoprotectants.
- In silico reconstruction of *P. freudenreichii* metabolism shows potential for GB biosynthesis from precursors like choline and GB aldehyde.
- Study of salt stress-induced protein variations is under way.