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

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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

Congrès RMN Biologique et les Enjeux du Vivant, Clermont-Ferrand, 12-14 mars 2003.

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

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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_2 production

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255 000 t in France, 1st 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

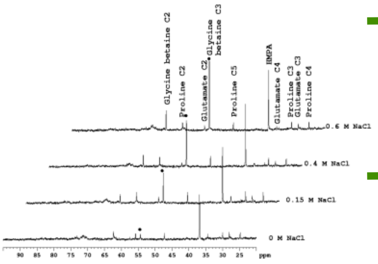
- Generally Recognized As Safe (GRAS status)
- Animal growth-promoting probiotic additive
- **Human probiotic**
- Apoptosis induction of colon cancer cells

(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 ^{13}C NMR data

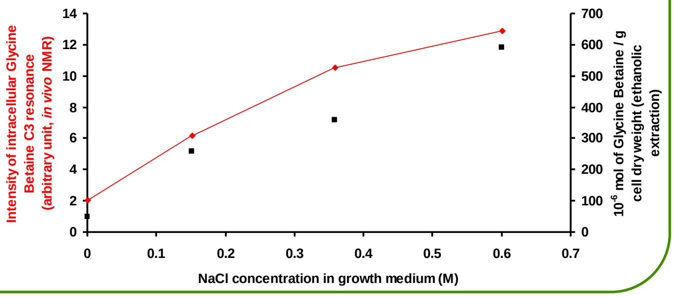


(512 scans (13 min), Bruker. Avance DMX 500, HMPA: reference in capillary)

In vivo natural abundance ^{13}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

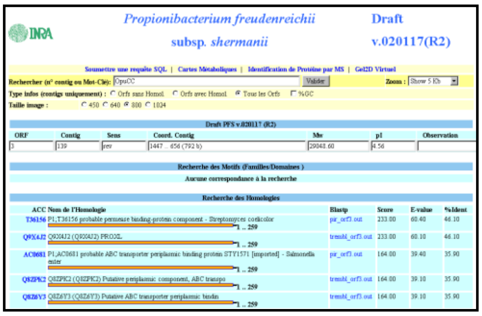
Genomic data (Sept. 2002)

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©, for efficient genomic annotation and *in silico* metabolic reconstruction.



Propionibacterium freudenreichii Draft
subsp. *shermanii* v.020117(R2)

Soumettre une requête SQL | Cartes Métaboliques | Identification de Protéine par MS | Gel2D Virtuel

Recherche (n° contig ou Mot-Clé): OpuCC Valider Zoom : Show 5 Kb

Type infos (contigs uniquement) : ○ Orfs sans Homol ○ Orfs avec Homol ● Tous les Orfs ☐ %GC

Taille image : ○ 450 ○ 640 ● 800 ○ 1024

Draft PFS v.020117 (R2)

ORF	Contig	Sens	Coord. Contig	Mw	pI	Observation
3	139	rev	1447 .. 656 (792 b)	29048.60	4.56	

Recherche des Motifs (Familles/Domaines)

Aucune correspondance à la recherche

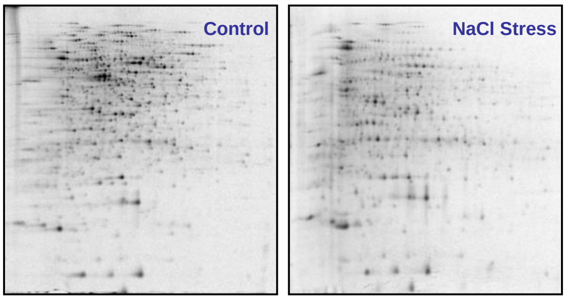
Recherche des Homologies

ACC	Nom de l'Homologie	Blastp	Score	E-value	%Ident
T36156	P1;T36156 probable permease binding-protein component - Streptomyces coelicolor 1 .. 259	pir_orf3.out	233.00	60.40	46.10
Q9X4J2	Q9X4J2 (Q9X4J2) PROXL 1 .. 259	trembl_orf3.out	233.00	60.10	46.10
AC0681	P1;AC0681 probable ABC transporter periplasmic binding protein STY1571 [imported] - Salmonella enter 1 .. 259	pir_orf3.out	164.00	39.40	35.90
Q8ZPK2	Q8ZPK2 (Q8ZPK2) Putative periplasmic component, ABC transpo 1 .. 259	trembl_orf3.out	164.00	39.10	35.90
Q8Z6Y3	Q8Z6Y3 (Q8Z6Y3) Putative ABC transporter periplasmic bindin 1 .. 259	trembl_orf3.out	164.00	39.10	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.

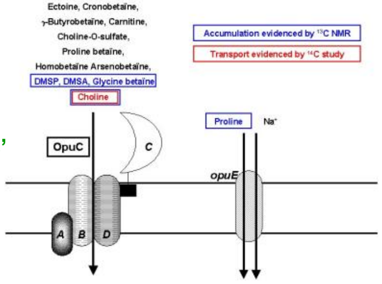
Proteomic data



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.