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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 CO₂ 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

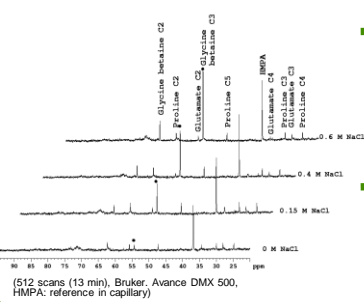
- Generally Recognized As Safe (GRAS status)
- Animal growth-promoting probiotic additive
- **Human probiotic**
- Apoptosis induction of colon cancer cells

(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

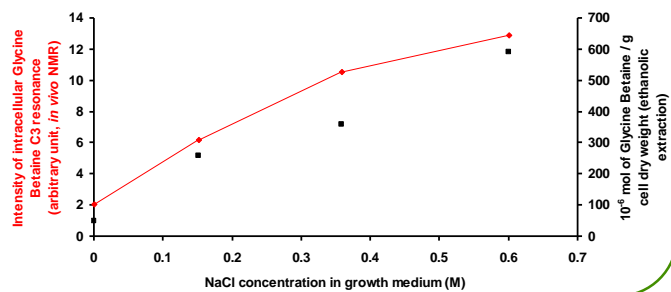
In vivo ¹³C NMR data



→ In vivo natural abundance ¹³C 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.

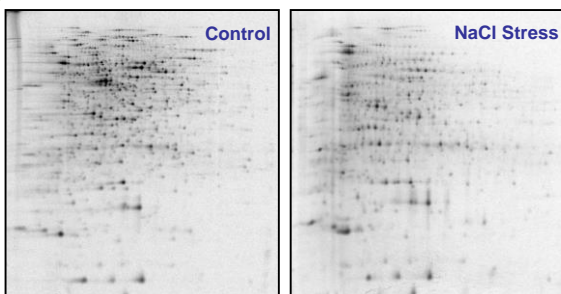
Gene	Contig	Size	Coord. Contig	Size	GC	Observation
ORF1	[13]	for	[1447-654(792 %)]	[2042 bp]	61.56	

ACC	Nom de l'homologue	BLAST	Score	E-value	%Ident
YMN4	PL_126156 prolyle personee binding protein; coagulation; -Drosophila melanogaster	pf_orf1.out	233.00	65.40	64.10
ORF4B	Q03612 (ORF4B) PRO-5	pf_orf1.out	223.00	65.10	64.10
ACM4B	PL_126163 prolyle ABC transport protein; binding protein; EF1571 (sequenced); -Salmonella enterica	pf_orf1.out	164.00	39.40	35.90
ORF2C	Q03613 (ORF2C) Prolyle personee binding protein; ABC transport; -Drosophila melanogaster	pf_orf1.out	164.00	39.10	35.90
ORF4A	Q03611 (ORF4A) Prolyle ABC transport protein; binding; -Drosophila melanogaster	pf_orf1.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.

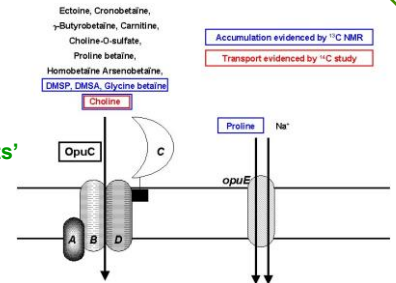
Proteomic data



→ 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.