

Evaluation of the diversity of two species of the genus *Propionibacterium* : Mass Spectrometry versus Triple-Locus Sequence Analysis

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SEVENTH FRAMEWORK PROGRAMME

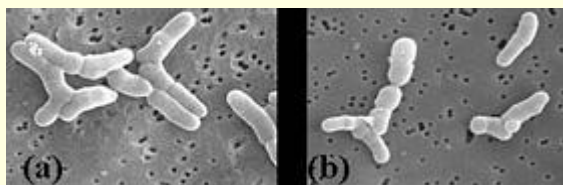
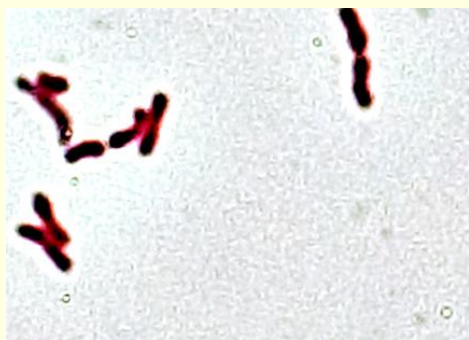
THEME: INFRA-2008-1.1.2.9

Biological Resources Centres (BRCs) for micro-organisms

GRANT agreement N° 228310

Genus *Propionibacterium*: phenotypic characteristics

- Gram-positive, rod-shaped bacteria that may bifurcate or even branch, nonspore-forming, anaerobic (microaerophilic)
- Generally catalase positive
- Characterized by the large amounts of propionic acid during growth



Propionibacterium freudenreichii and *Propionibacterium acnes*: Habitat and Functions

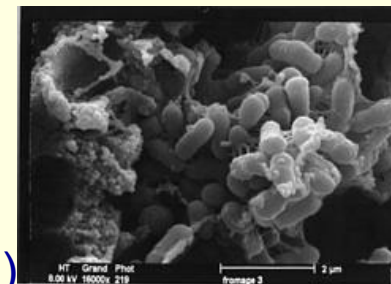
Propionibacterium freudenreichii:

- mainly isolated from dairy sources
- important function in the cheese industry involved in the hole and flavor formations in Swiss-type cheese
- probiotic potential: production of vitamin B12 and inhibition of the unwanted microflora



Propionibacterium acnes:

- isolated from human (commensal of the skin, mouth...)
- opportunistic pathogen (acne, endocarditis, prosthetic joint infections...)



Aims of the study

- To determine the degree of molecular diversity within *P. freudenreichii* and *P. acnes* at species and clonal level
- To compare the ranking of strains according to the methodology applied : triple locus sequencing or mass spectrometry
- To evaluate the reproducibility of the mass spectrometry method (two equipments, two laboratories and two data analysis methods)

Materials

20 strains of *P. freudenreichii* from CIRM-BIA, INRA selected in order to offer a large diversity in terms of biotope (geographic and habitat)

→ 2 subspecies (*freudenreichii* and *shermanii* described on the basis of lactose fermentation and nitrate reductase activity)

Origin	<i>P. freudenreichii</i> subsp. <i>freudenreichii</i>	<i>P. freudenreichii</i> subsp. <i>shermanii</i>
raw milk	/	6
cheese	1	8
"vegetal" (hay, straw, wheat)	/	4
unknown	/	1

21 strains of *P. acnes* from Institut Pasteur isolated from human between 1920-1960 and 2000-2009

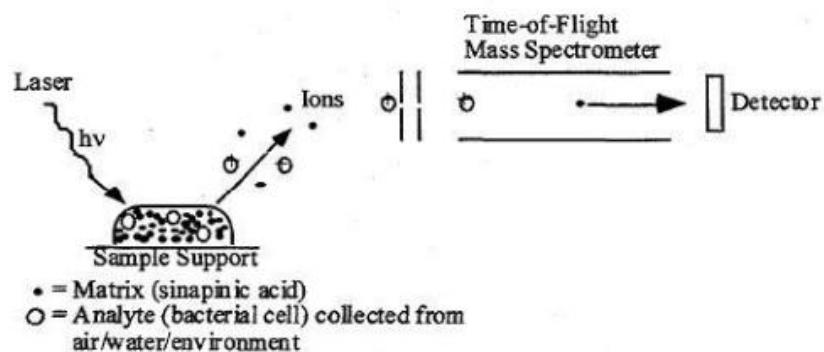
→ 3 phylogenetic groups described by sequence analysis of *recA* and *tly* genes: Types I, II, III

Origin	<i>P. acnes</i>
blood	7
Various (acne, abscess..)	9
unknown	5

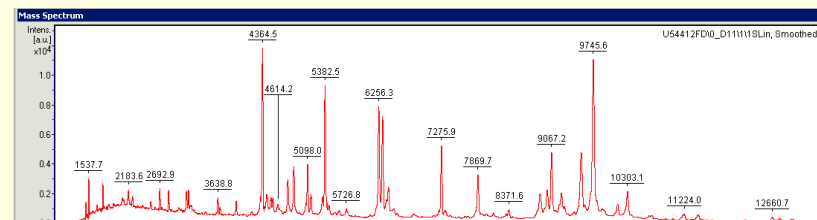
Methods

- Gene sequencing: *P. acnes*: 16S rRNA, *rpoB*, *adh*, *gyrB*
P. freudenreichii: 16S rRNA, *rpoB*, *adh*, *fumC*
- MALDI-TOF Mass Spectrometry

- Based on mass analysis of protein composition of the bacterial cells
- Generation of spectra



(Wahl et al. 1999)

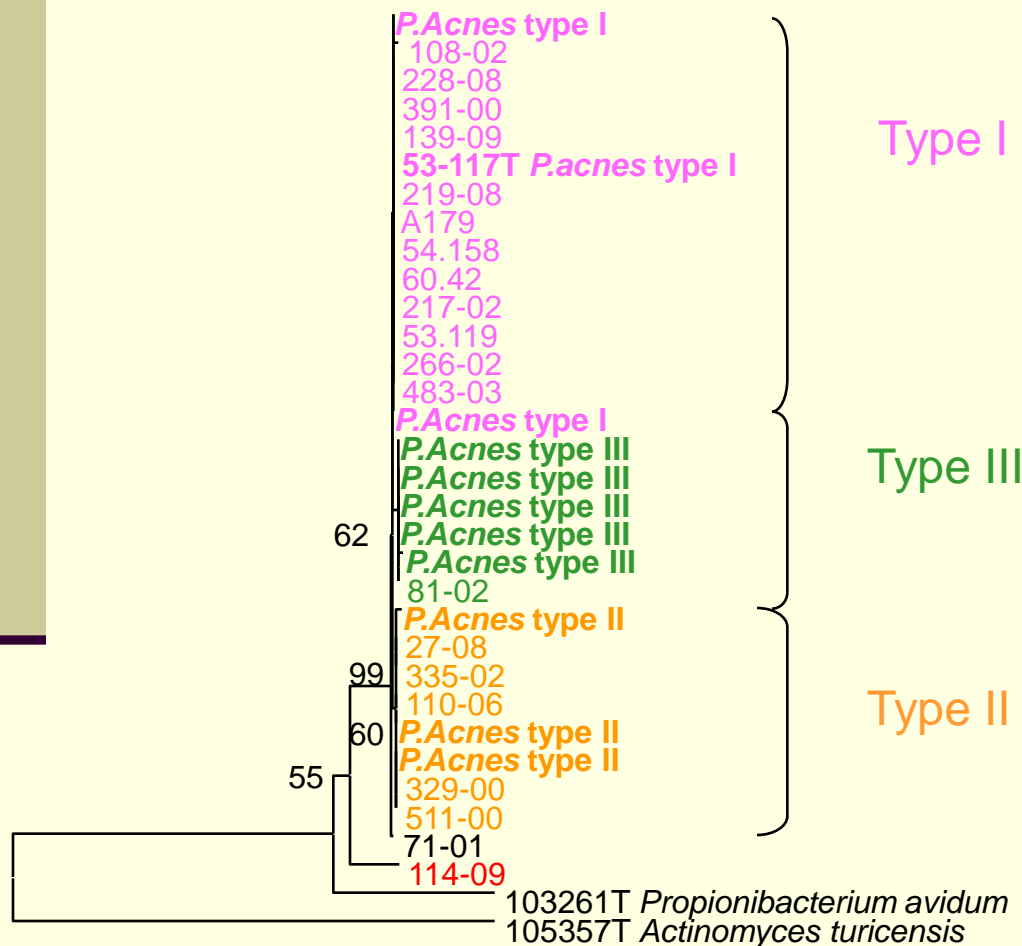


Bruker Daltonics autoflex (IP):
 Comparison with high quality reference spectra, generated by culture collections



Applied Biosystems equipment (INRA/CIRM):
 Statistical analysis of the data:
 → Principal component analysis

Results: Phylogenetic tree based on 16S rRNA gene sequences for *P. acnes*



Type I

Type III

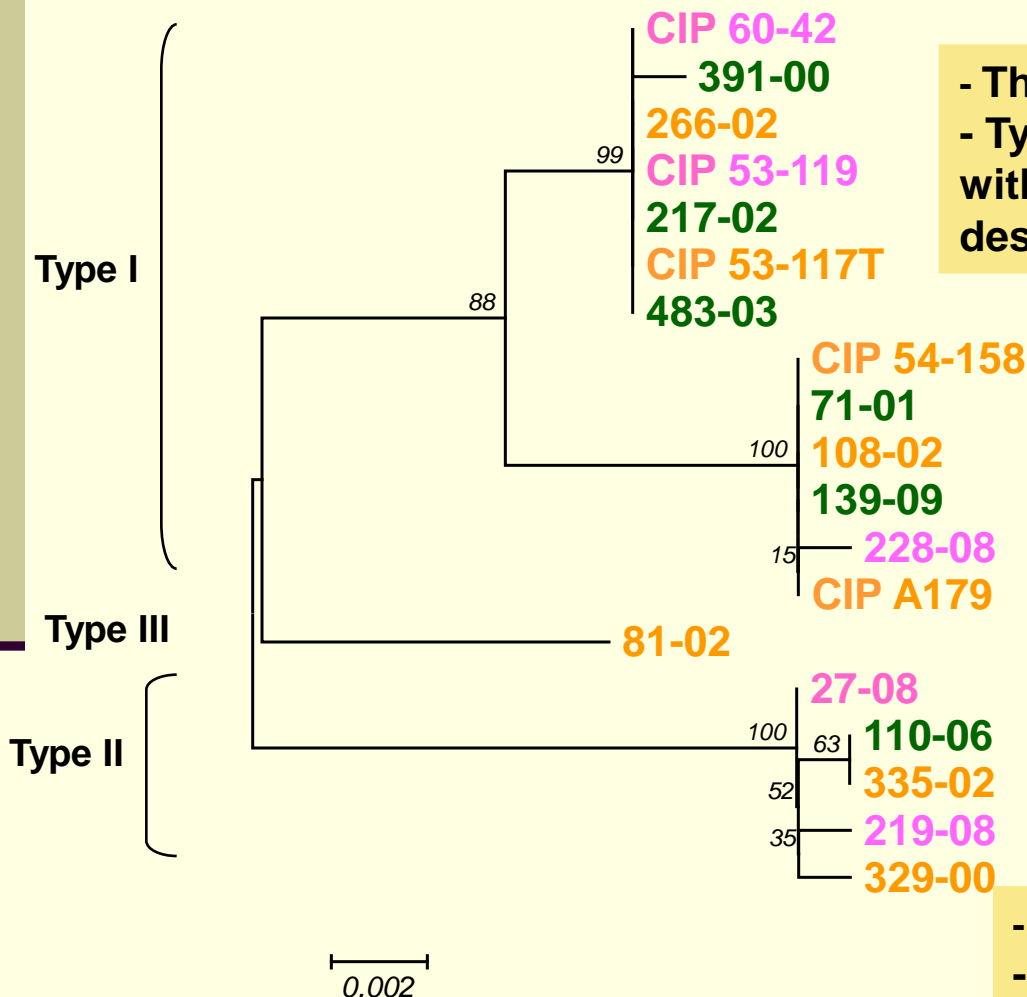
Type II

- The 3 types previously described are found among the strains studied.
- The place of strain 71-01 is not well defined by 16S rRNA gene.
- 114-09 is distant from other strains. Result was confirmed by using the genes *rpoB*, *gyrB* and *adk*.

Control strains of the 3 types indicated in bold

- *A. turencis* was used as outgroup. Neighbour-joining method.
 - Numbers represent bootstrap values of 100 replicates

Phylogenetic tree based on combined *adk*, *rpoB* and *gyrB* gene sequences for *P. acnes*

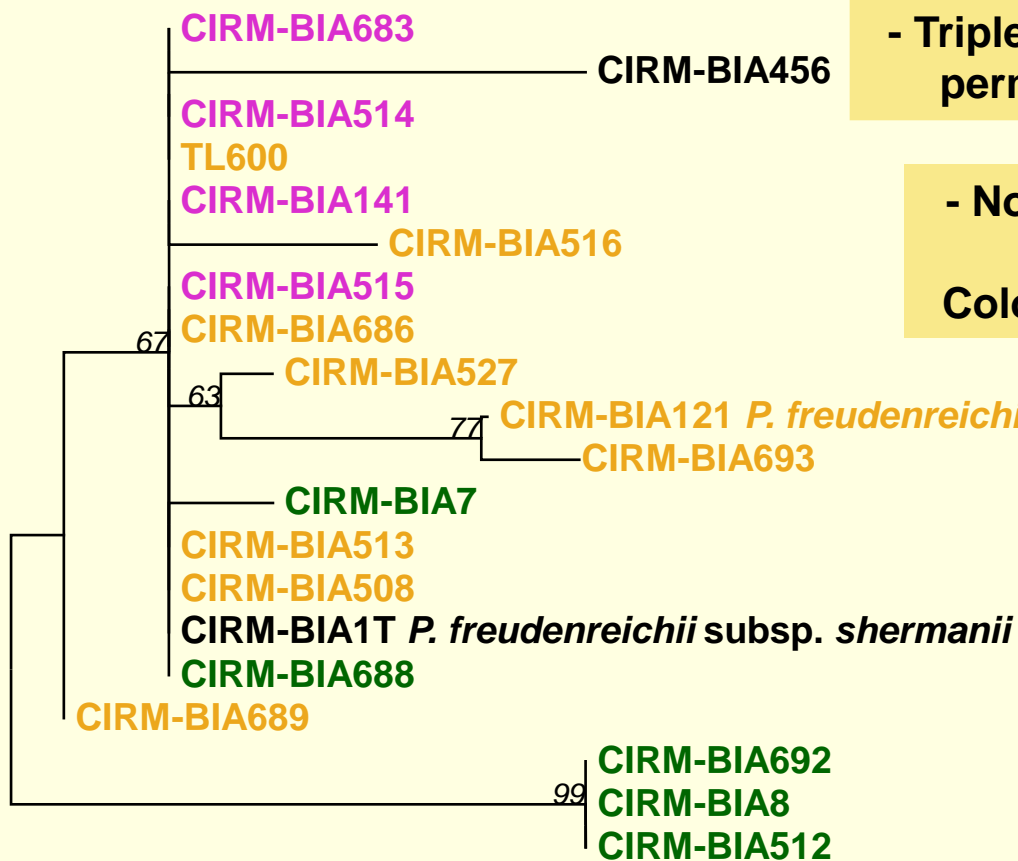


- The 3 types are well defined
 - Type I contains 2 groups not seen with 16S rRNA and not already described.

- No relation between strain origins and clusters.
 Colour Code : **blood**, **various**, **unknown**

- All the old strains belong to Type I
 - No Type specificity for the strains recently isolated.

Phylogenetic trees based on combined *adk*, *rpoB* and *fumC* gene sequences for *P. freudenreichii*



DNA-DNA homology within the *P. freudenreichii* species is very high (more than 86%)

- Triple locus sequence analysis does not permit to separate the 2 subspecies.

- No relation between strain origin and clusters.

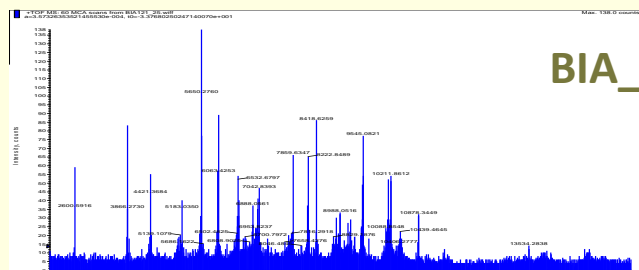
Colour Code : Cheese, raw milk, wheat

Results confirmed by MLST study of 7 genes on 100 strains.

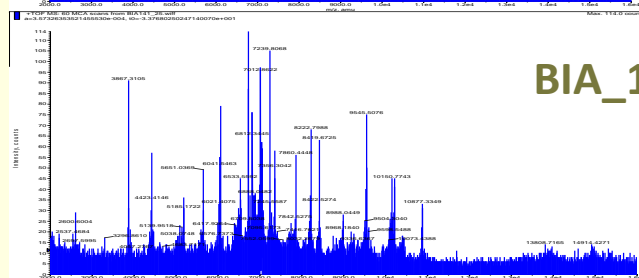
See Area 2 - Poster Number: 17
 "Lineages with broad dairy biotope ranges and phenotypic variability in *Propionibacterium freudenreichii* revealed by multilocus sequence typing"

MALDI-TOF results: examples of profiles

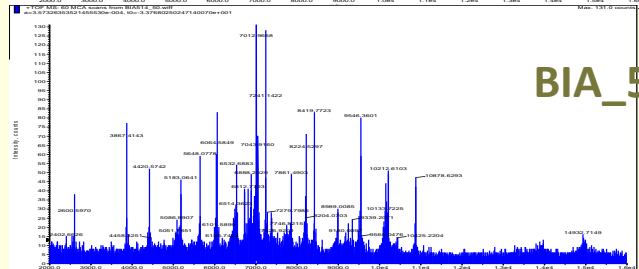
P. freudenreichii



BIA_121

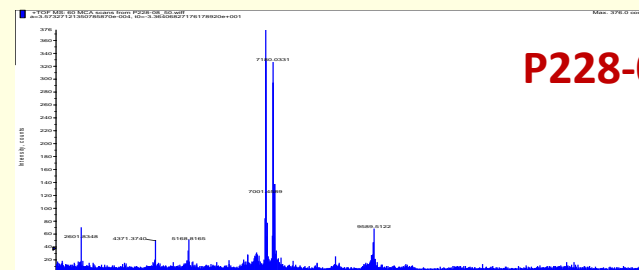


BIA_141

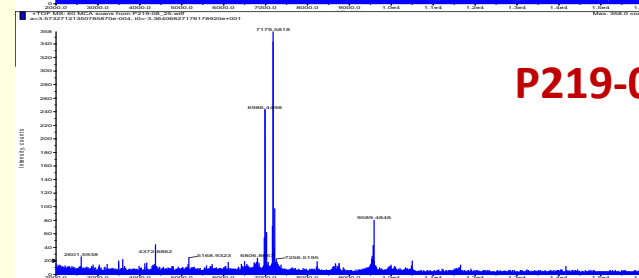


BIA_514

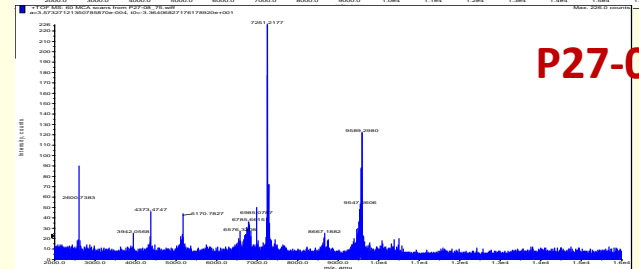
P. acnes



P228-08



P219-08

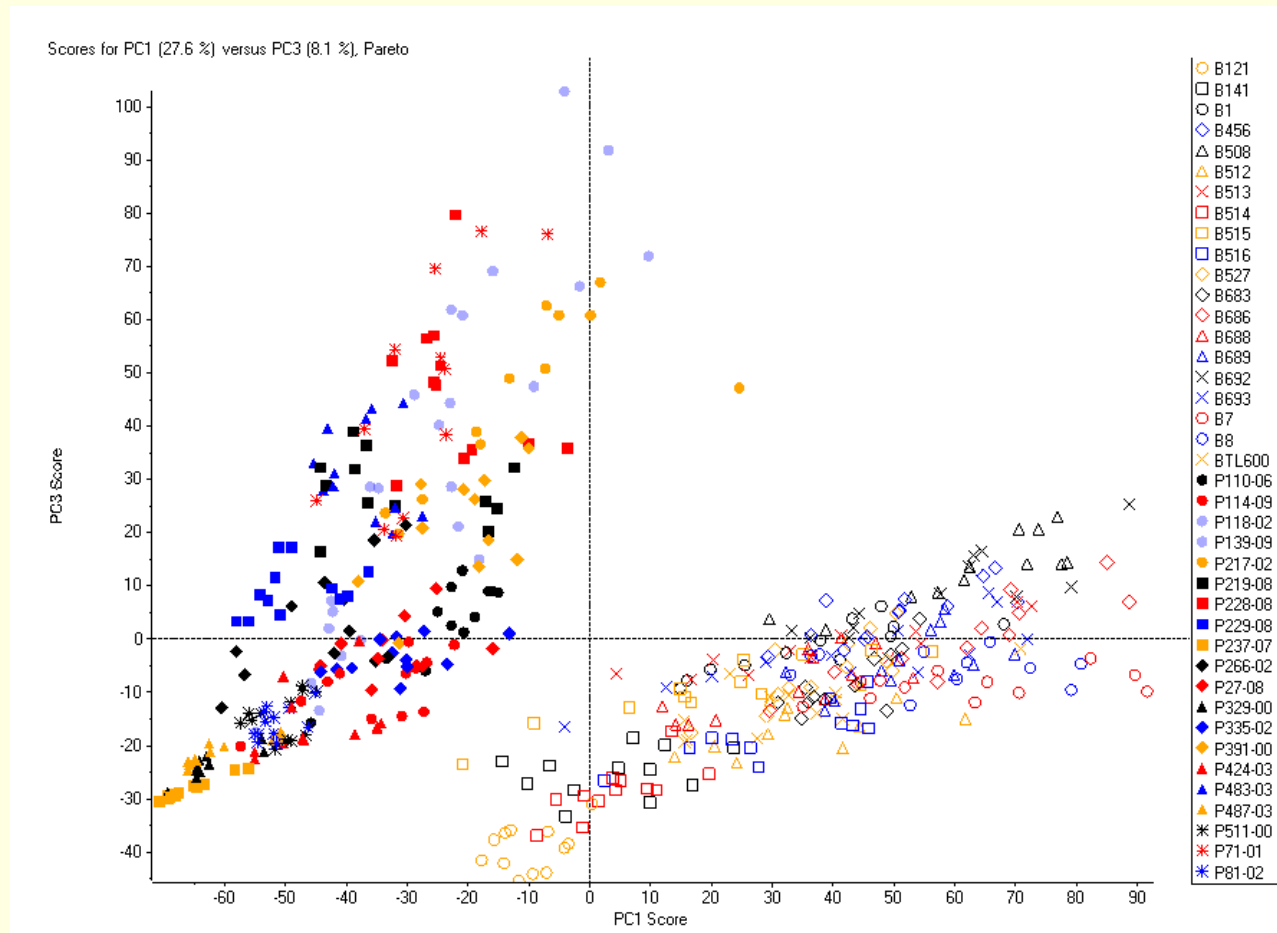


P27-08

2 types of profiles in relation with the species of *Propionibacterium*

→ One species = one specific profile

MALDI-TOF results: Principal Component Analysis



**12 spectra
obtained for
each strain**

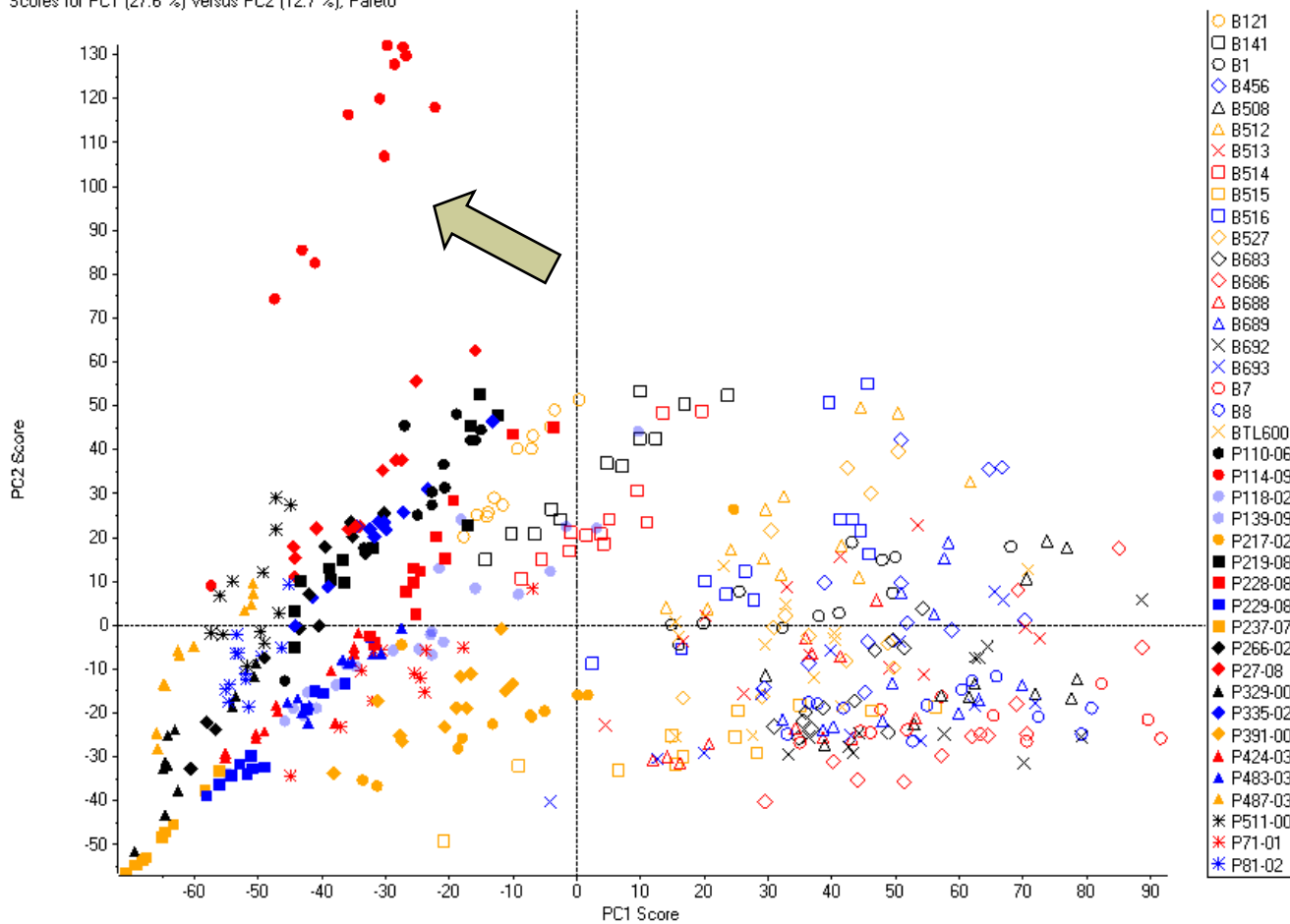
2 groups corresponding to the 2 species *P. freudenreichii* and
P. acnes

MALDI-TOF results:

Principal Component Analysis: strain 114-09

20100921_CIRM_480_spectres_analyse2

Scores for PC1 (27.6 %) versus PC2 (12.7 %), Pareto

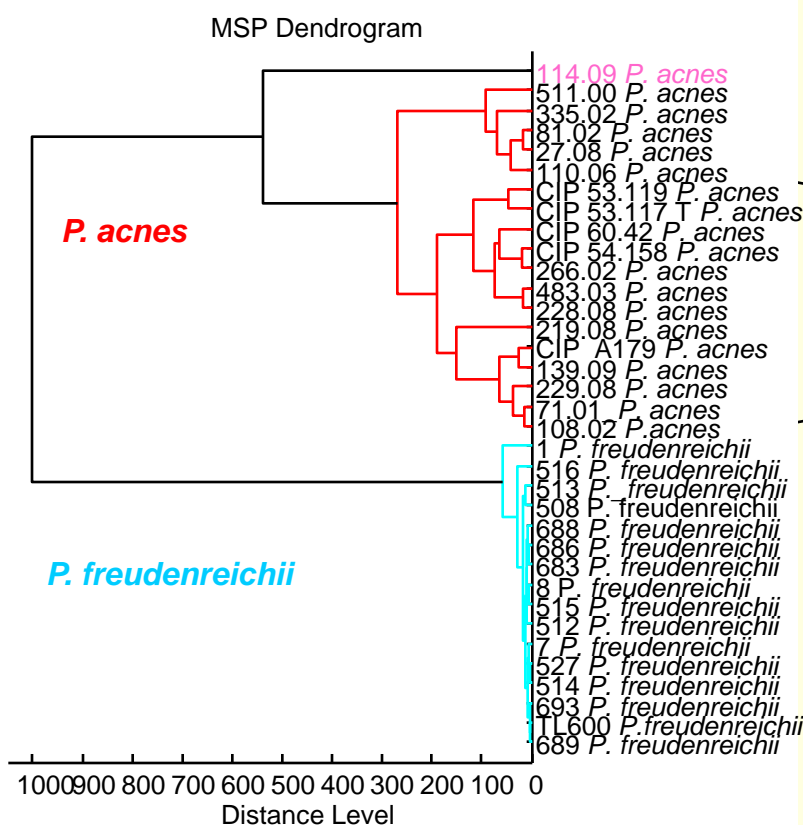


12 spectra
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each strain

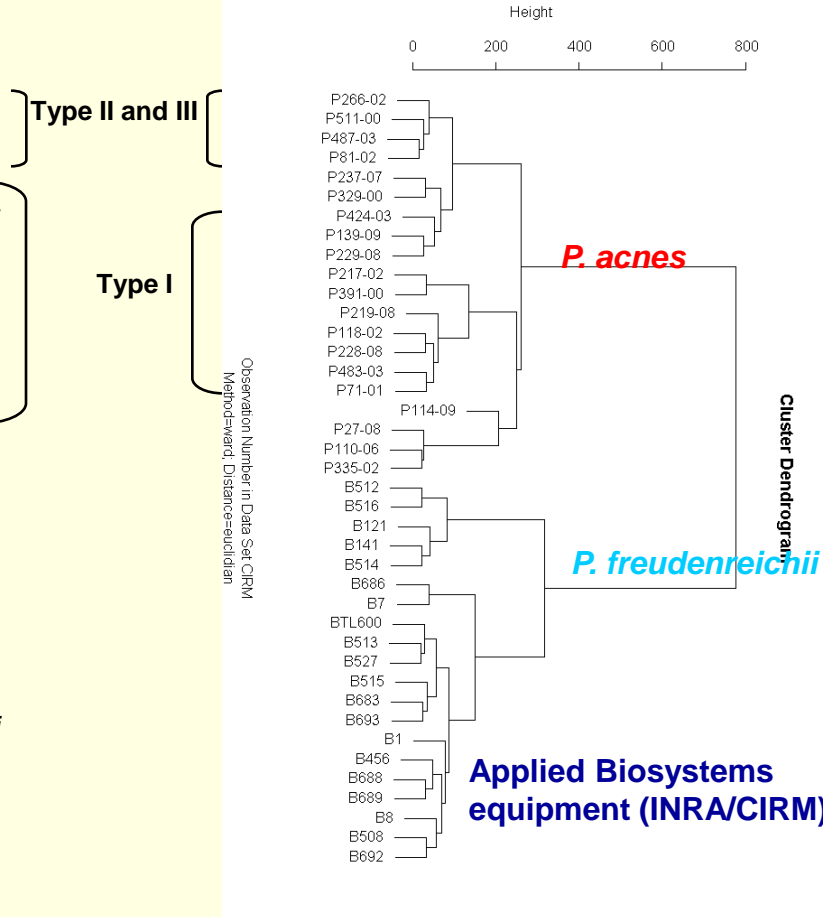
Strain 114-09 : away from other strains according to phylogenetic trees based on 16S rRNA and genes *rpoB*, *gyrB* and *adk*, it also shows an atypical mass profil

MALDI-TOF results:

Comparison between the two equipments used



Bruker Daltonics autoflex (IP)



- Similar results obtained whatever the equipment and analyse type performed
- Regarding *P. acnes*, the groups obtained obviously reflect the molecular types

Conclusions (1)

- Congruence between individual housekeeping gene trees.
- No phylogenetic resolution of the two *P. freudenreichii* subspecies with *adk*, *rpoB*, and *fumC* genes.
- An other type could exist in *P. acnes* based on *gyrB*, *adk* and *rpoB* analysis.
Is the discriminatory power of *gyrB*, *rpoB*, and *adk* higher for *P. acnes* compared to that of gene *recA*?
- Genetic diversity in *P. freudenreichii* seems to be more important than in *P. acnes*.

Conclusions (2)

- A good reproducibility was obtained with mass spectrometry: Identification by mass spectrometry analysis is not dependent on the equipment and also not dependent on the data analysis methodology.
- Concerning *P. acnes*, the 3 types previously described were for a majority also found with mass spectrometry analysis.
- Example of CIP 114-09 which has an atypical molecular profile also has an atypical mass spectrometry profile.

→ These results underline the strength of the mass spectrometry method for taxonomy purpose