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 Nº 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:**
- 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)
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)

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

<table>
<thead>
<tr>
<th>Origin</th>
<th><em>P. freudenreichii</em> subsp. <em>freudenreichii</em></th>
<th><em>P. freudenreichii</em> subsp. <em>shermanii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>raw milk</td>
<td>/</td>
<td>6</td>
</tr>
<tr>
<td>cheese</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>&quot;vegetal&quot; (hay, straw, wheat)</td>
<td>/</td>
<td>4</td>
</tr>
<tr>
<td>unknown</td>
<td>/</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin</th>
<th><em>P. acnes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>7</td>
</tr>
<tr>
<td>Various (acne, abscess..)</td>
<td>9</td>
</tr>
<tr>
<td>unknown</td>
<td>5</td>
</tr>
</tbody>
</table>
Methods

- Gene sequencing: *P. acnes*: 16S rRNA, *rpoB*, *adk*, *gyrB*
  *P. freudenreichii*: 16S rRNA, *rpoB*, *adk*, *fumC*

- MALDI-TOF Mass Spectrometry
  - Based on mass analysis of protein composition of the bacterial cells
  - Generation of spectra

Bruker Daltonics autotof (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*

- 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 \textit{adk}, \textit{rpoB} and \textit{gyrB} gene sequences for \textit{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: \textbf{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

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

→ One species = one specific profile
MALDI-TOF results:
Principal Component Analysis

2 groups corresponding to the 2 species *P. freudenreichii* and *P. acnes*
MALDI-TOF results:
Principal Component Analysis: strain 114-09

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

- Similar results obtained whichever 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.