



Development of an omic biochip-like for the detection & identification of sporeforming bacteria in food

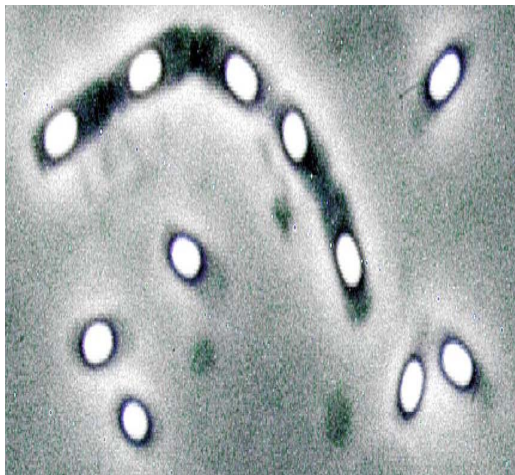
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Sporeformers in food

- Endospore with extreme resistance & high adhesion to surfaces
- Food safety & economical issue: food poisoning & food spoilage
- Sporeformers are:
 - widespread in the environment (ground, water...)
 - aerobic, facultative anaerobic or strictly anaerobic
 - psychrophiles, mesophilic or thermophiles
- Evolving taxonomy & phylogenetic relationship (gene sequencing)



- *Bacillus*, *Alicyclobacillus*, *Paenibacillus*, *Brevibacillus*,
Aneurinibacillus, *Virgibacillus*, *Gracilibacillus*,
Salibacillus, *Geobacillus*, *Ureibacillus*, *Lysinibacillus* ...

- *Clostridium*, *Moorella*, *Dendrosporobacter*,
Thermoanaerobacter, *Thermoanaerobacterium*,
Desulfotomaculum, *Caloramator* ...

- ...



Sporeformers in food: industrial issue

Sporeforming bacteria: unintended food contaminants
Adaptation or selection by food processes?



Lack of fast & specific diagnosis
tool to investigate sporeformers &
encountered prevalence in food



Partnership

- Industrial partners

BBA (9 industrial groups)



ADROuest (6 industrial groups)



3 industrial groups



- Scientific partners



Primer-probe design
GeneDisc conception

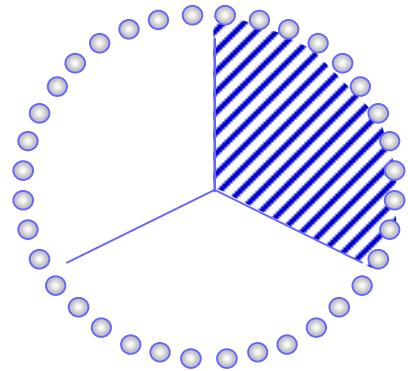




Developped GeneDiscs

- 2 GeneDiscs have been developped for the analyse of 3 samples in parallel
 - targeted organisms: prevalence & recognised spoilage activities
 - primer/probe: housekeeping genes & genes encoding metabolic activity (NCBI)

✓ GeneDisc1 « Genus »



- *Bacillus*
- *Paenibacillus*
- *Clostridium*
- *Moorella*

- *Geobacillus stearothermophilus*
- *Anoxybacillus flavithermus*

- PCR controls

✓ GeneDisc2 « *Bacillus spp.* »

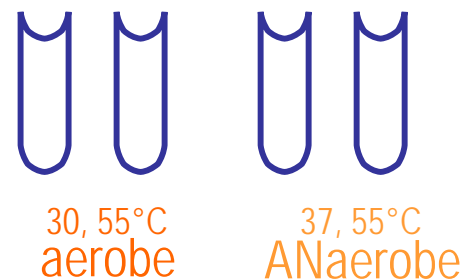
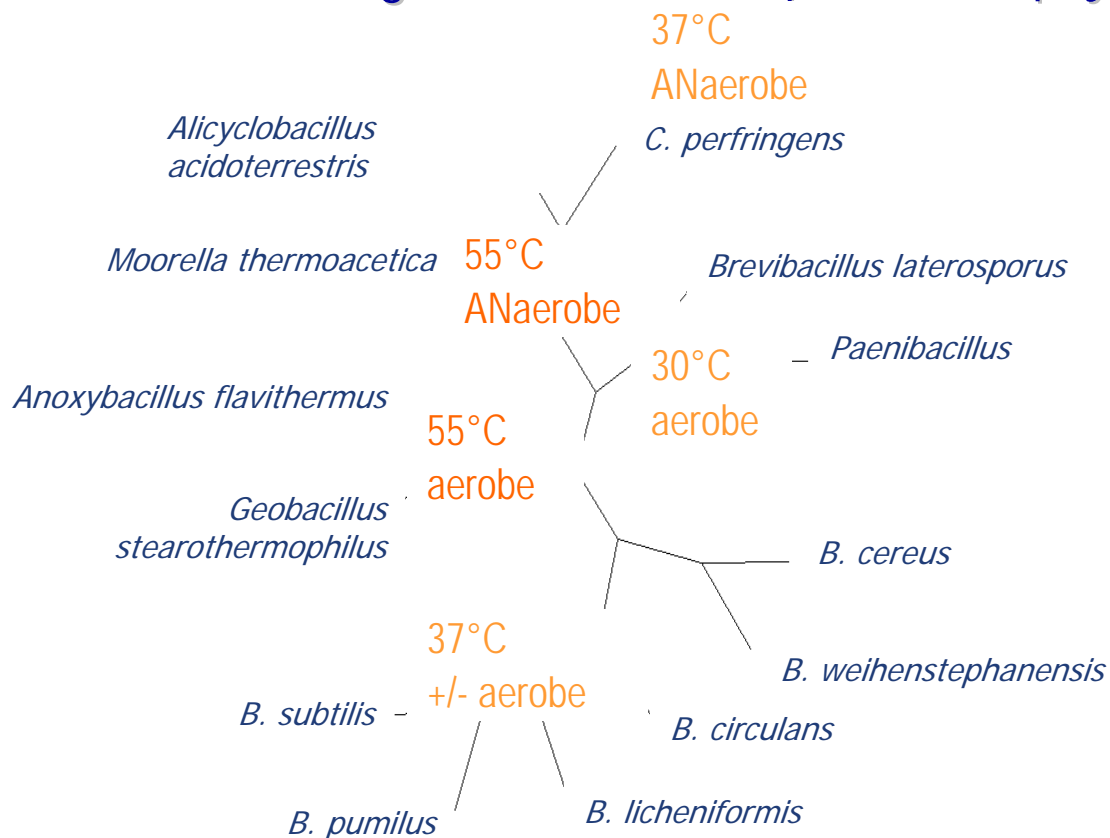
- Genus *Bacillus*
- *B. licheniformis*
- *B. pumilus*
- *B. sporothermodurans*
- *B. subtilis*
- *B. cereus* / *B. thuringiensis*
- *B. weihenstephanensis* / *B. mycooides*
- *Brevibacillus laterosporus*

- PCR controls



Food enrichment

- Overnight enrichment step: sporeformers biodiversity recovery
- Wide range of nutritional requirements, physiological and metabolic diversity



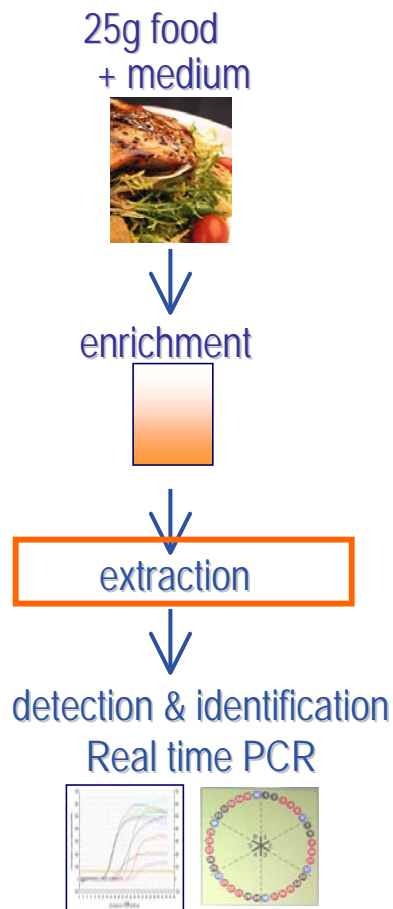
➤ enrichment >10⁶CFU/ml in food



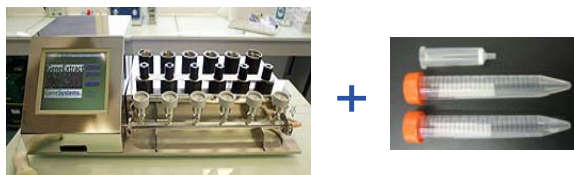
Food extraction

➤ 3 extraction protocols to further simplify the use of the developed tools

➤ objectives: fast, reliable and low cost biochip
... easy to use in microbiology lab

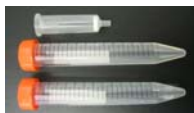


1. Protocol with « GeneExtract »: 120 min



| Lysis | Purification |
|-----------------------------------|--------------|
| chemical mechanical thermal | yes |

2. Protocol with « spin column »: 90 min



| | |
|---------------------|-----|
| chemical thermal | yes |
|---------------------|-----|

3. Protocol with « chelex beads »: 45 min



| | |
|---------|----|
| thermal | no |
|---------|----|



GeneDisc specificity

- GeneDisc specificity: ability to detect targeted cells (inclusivity) & not non-targeted cells (exclusivity) ...
- DNA from more than 220 collected strains
- Collected strains mostly composed of food isolates (38 species)
- Inclusivity: all targeted species are detected
- Exclusivity : no cross-reaction observed



GeneDisc detection limit

➤ GeneDisc detection limit (*B. cereus*)

- Artificial contamination levels: 100, 50, 10, 5, 1, 0 spores of *B. cereus*/25g food
- Detection limit evaluated by standard method / GeneDisc
- Calculation of 50% endpoint limit of detection (LOD_{50}) & confidence intervals (95%) by Spearman Kärber test (ISO validation) → statistical evaluation of detection limit

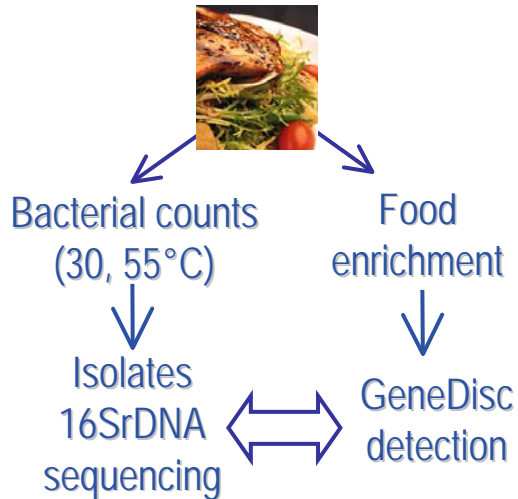
| food matrix | detection level (sp/g) | |
|--------------|------------------------|----------------------|
| | Standard | GeneDisc |
| desert cream | 8.35 [4.014; 17.381] | 0.036 [0.016; 0.08] |
| couscous | 8.53 [2.455; 29.663] | 0.072 [0.031; 0.178] |
| fish soup | 8.805 [4.231; 18.322] | 0.017 [0.01; 0.037] |
| egg white | <10 | 0.163 [0.068; 0.392] |
| whole egg | <10 | 0.098 [0.030; 0.316] |

→ *B. cereus* detection limits with GeneDisc < 1sp/g food or about 2sp/25g food



GeneDisc accuracy

➤ Validation study



- Naturally contaminated food: 60 food products
- Artificially contaminated food: 152 contamination conditions
3 category food products

➔ GeneDisc detection is confirmed by sporeformer colonies

➔ Sporeformer prevalence in ingredients & along food production line

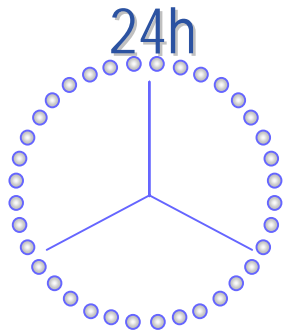
➔ Identification of other sporeformer genus: *Thermoactinomyces*, *Ureibacillus*, *Aneurinibacillus*, *Lysinibacillus*, *Dendrosporobacter*...

... Study of prevalence encountered in food industry continues (Memospore 2008-2010) ...



Future work

➤ GeneDiscs performances



- Detection 6 genus, 9 species
- Specific, sensitive & accurate

➤ collaborative trial to evaluate reliability & practicability of the GeneDiscs

➤ GeneDiscs optimisation, update & marketing ...

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