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Use of principal component analysis in order to link genotypic and phenotypic characters of psychrotrophic *Bacillus cereus* group isolates to their cytotoxic and spoilage potentials in egg-breaking industry

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Microbial stabilization of liquid egg products in France

- Egg products are used in susceptible food like cream, dessert, dairy, meat and seafood products

Breaking

Filtration →

Pasteurization

Cooling →

Conditioning
Storage
Delivery

Whole egg and egg yolk:
55°C to 65°C for 2 to 6 min
Egg white:
55°C to 57°C for 2 to 6 min

Refrigeration:
From few weeks to 3 months

↓

Destruction of major vegetative cells

Inefficient on spore forming bacteria

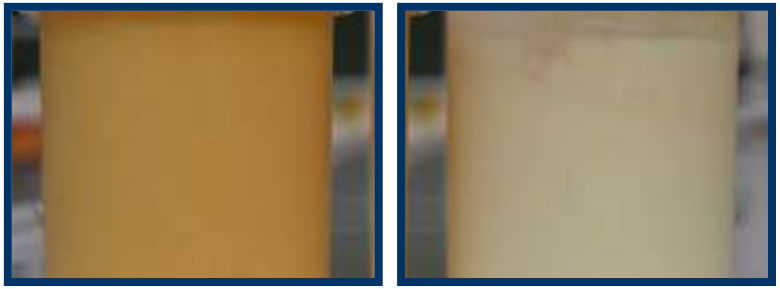
↓

Selection of a psychrotrophic microbiota

Bacteria of the *Bacillus cereus* group in liquid egg products

Properties of *B. cereus* group cells:

- ➜ Ubiquity
- ➜ Sporulation
- ➜ Adhesion
- ➜ Psychrotrophy
- ➜ Production of enzymes and/or toxins



Safe egg product

Spoiled egg product

Foodborne illness

Egg product spoilage

⇨ Economic and/or public health problems

Origin of the collection of *B. cereus* group isolates

Collection of liquid whole egg samples
in 6 companies

Warm season:
May 07 → Sept 07

Cold season:
Nov 07 → Febr 08

Enrichments at 10°C for 5 days
in peptone water with 5 g/L LiCl → To favour the development of psychrotrophic isolates

Detection of *B. cereus* group bacteria isolates
(*sspE* signature assayed by Real-time PCR,
according to Kim *et al.*, 2005)

Typing by ERIC-PCR and PFGE

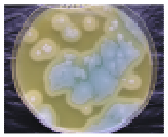
77 isolates

Characterization and behavior of the 77 isolates

Characterization of psychrotrophic isolates, according to the literature:

- Presence of genetic signatures: *cspA* (Francis *et al.*, 1998)
  16S rDNA-2p (Von Stetten *et al.*, 1998)
  16S rDNA-1m (Von Stetten *et al.*, 1998)

- Ability to grow at 6°C and 43°C on solid medium (Lechner *et al.*, 1999)



Study of the food poisoning and egg product spoilage abilities:

- Growth ability at 6°C, 10°C, and 30°C in BHI-YE broth and in liquid whole egg
  ⇨ cell enumeration (Log cfu/mL) at different times
- Cytotoxic activity at 10°C and 30°C in BHI-YE broth and in liquid whole egg
  ⇨ percentage of inhibition of Caco2 cells
- Egg spoilage ability at 10°C and 30°C
  ⇨ time of liquid whole egg coagulation

Characterization of the isolates

Profile	*cspA*	*16S rDNA-1m*	*16S rDNA-2p*	Growth at 43°C	Growth at 6°C	Nb isolates	% isolates	
1	1	0	1	0	1	35	45.5	
2	0	1	1	0	1	3	3.8	
3	0	1	1	1	1	12	15.6	?
4	0	1	1	1	0	4	5.2	
5	0	1	1	0	0	3	3.9	
6	0	0	1	1	1	6	7.8	?
7	0	0	1	0	1	5	6.5	
8	0	0	1	0	0	2	2.6	
9	0	0	1	1	0	2	2.6	
10	1	0	1	1	0	1	1.3	?
11	1	0	1	1	1	2	2.6	?
12	1	1	1	1	0	2	2.6	?

12 profiles , all have the 16s rDNA-2p signature

Profile 1: *B. weihenstephanensis* (Lechner *et al.*, 1998), main profile

5 profiles not described in the literature (3, 6, 10, 11, 12)

Global analysis of the isolates in egg product environment

Genotypic and phenotypic signatures: 12 profiles
Growth ability
Cytotoxic activity
Egg spoilage ability

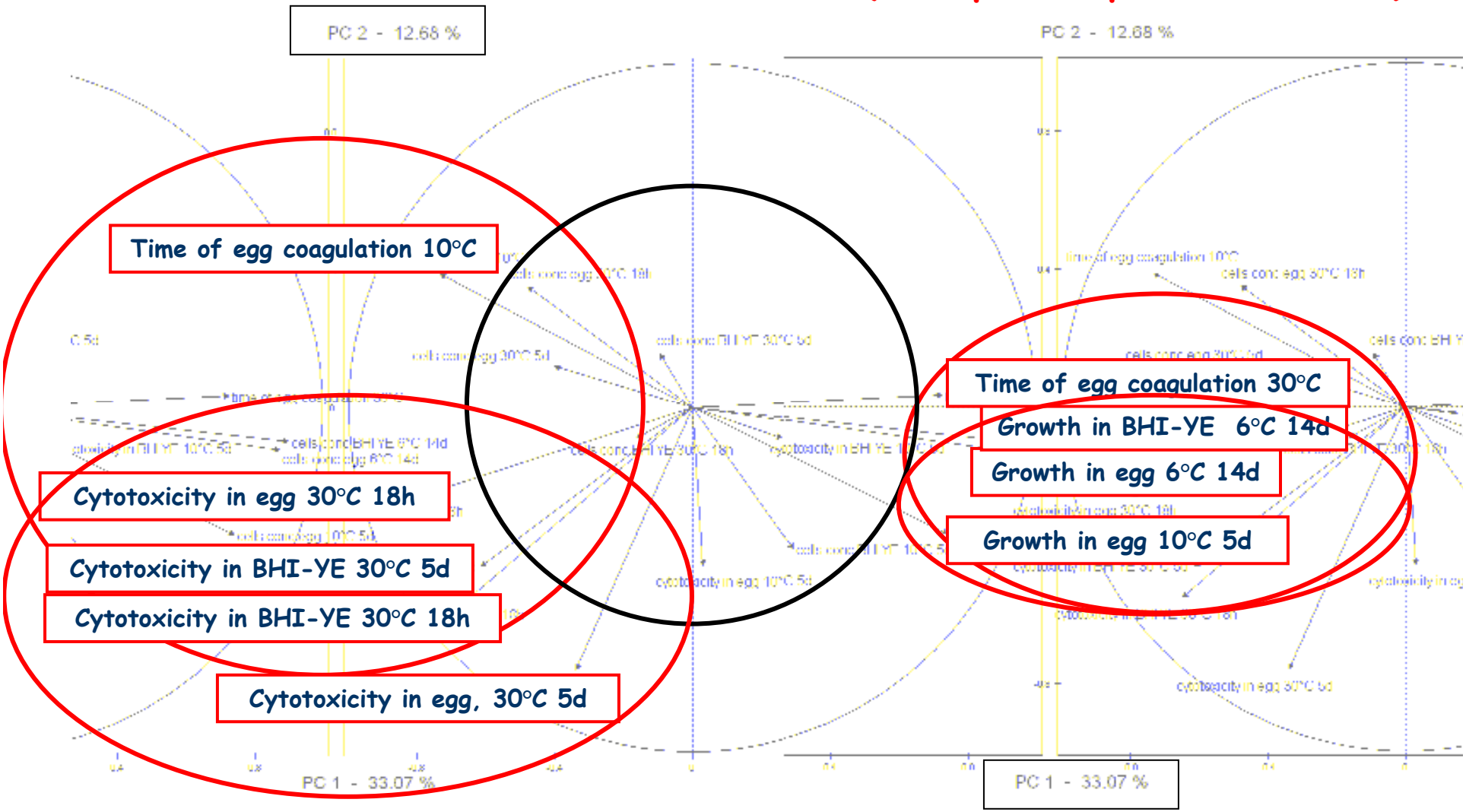
} links ?

Global behavior of the isolates with respect to
(i) their origin (6 companies) ?
(ii) the season (warm/cold) ?

→ **Principal Component Analysis**

Global analysis of the isolates in egg product environment

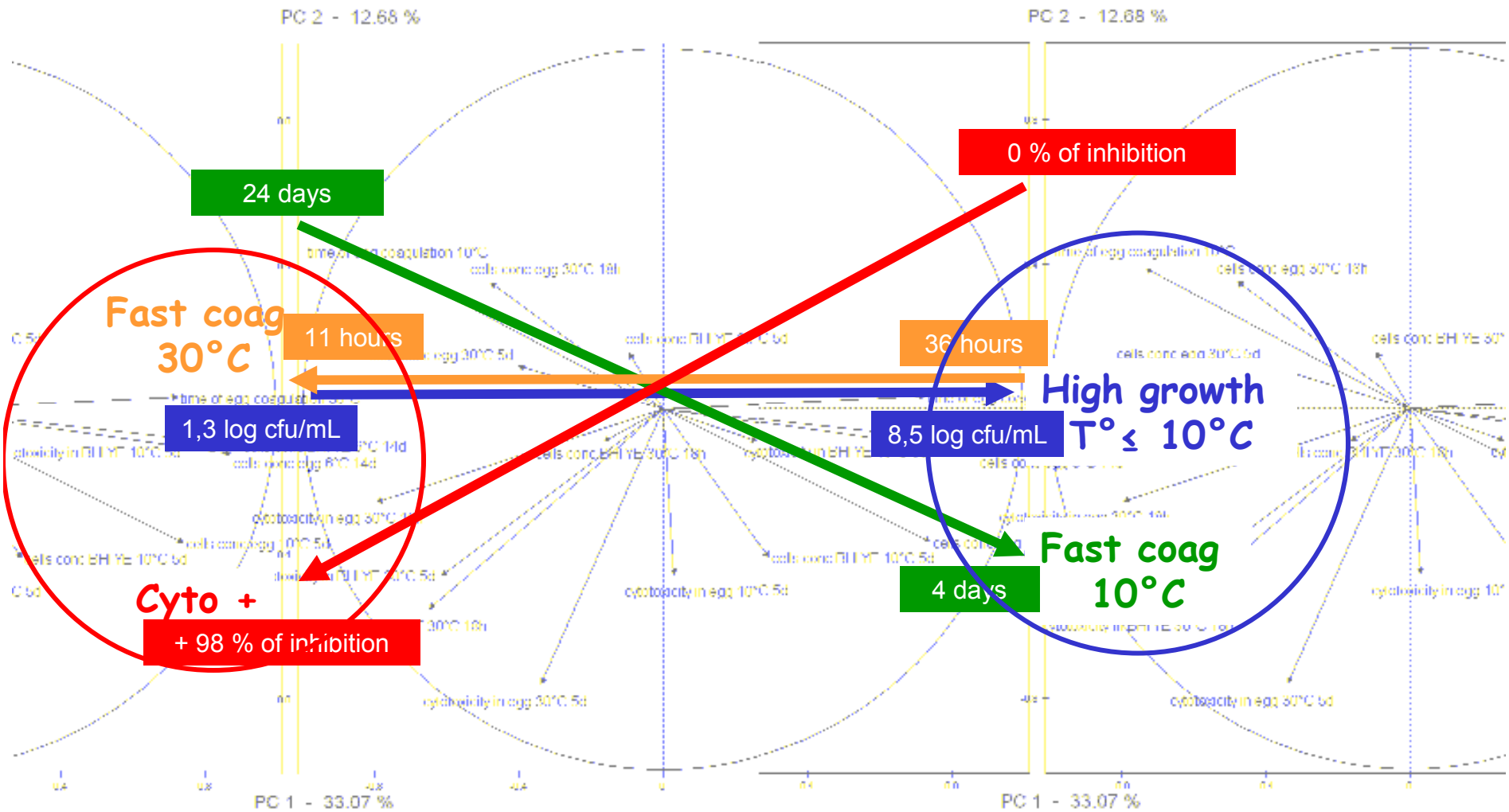
Correlation circle of the continuous variables (Principal Components 1 and 2)



- Liquid whole egg = BHI-YE for growth (≤ 10°C) and for toxin production at 30°C

Global analysis of the isolates in egg product environment

Correlation circle of the continuous variables (Principal Components 1 and 2)



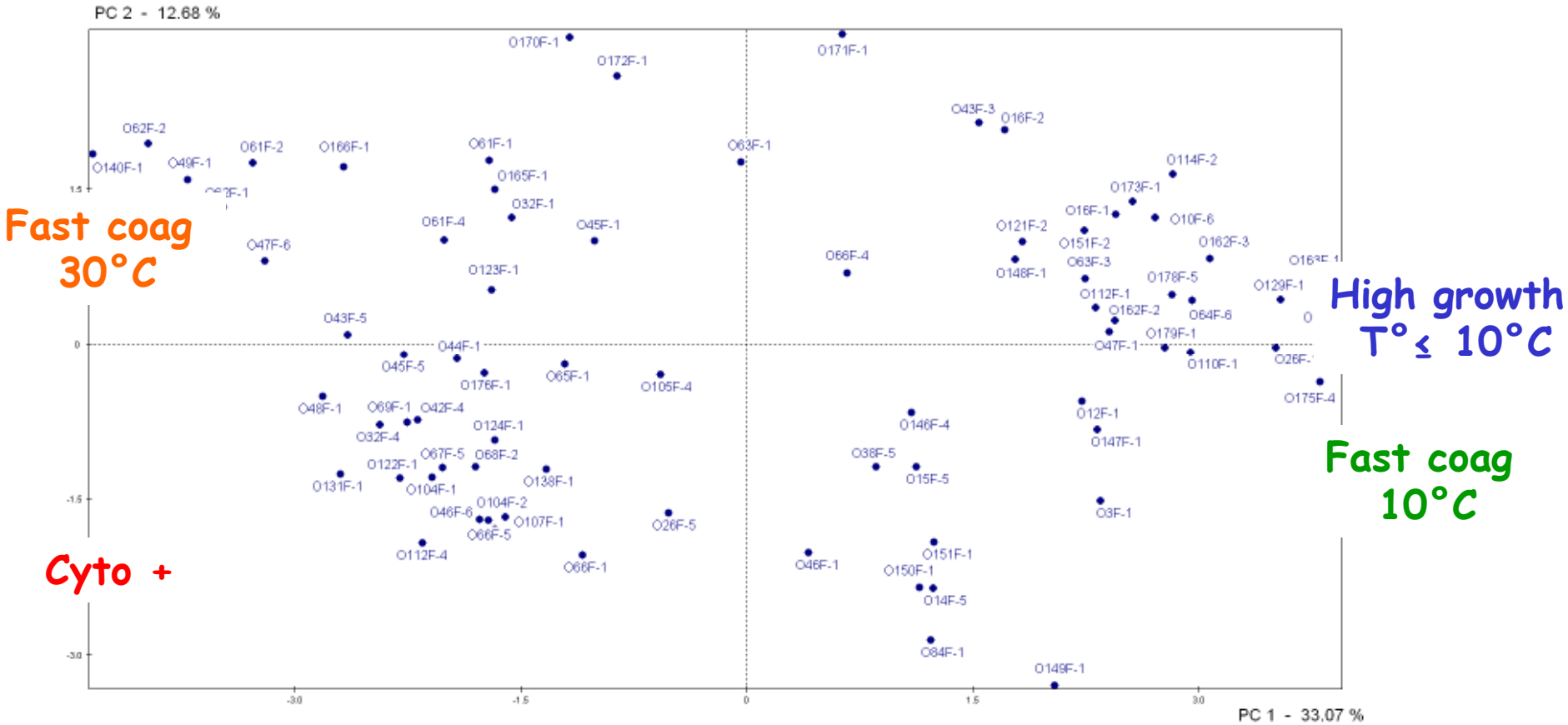
- High cytotoxic activity at 30°C
  Fast egg coagulation at 30°C

≠

High growth ability at ≤ 10°C
Fast egg coagulation at 10°C

Global analysis of the isolates in egg product environment

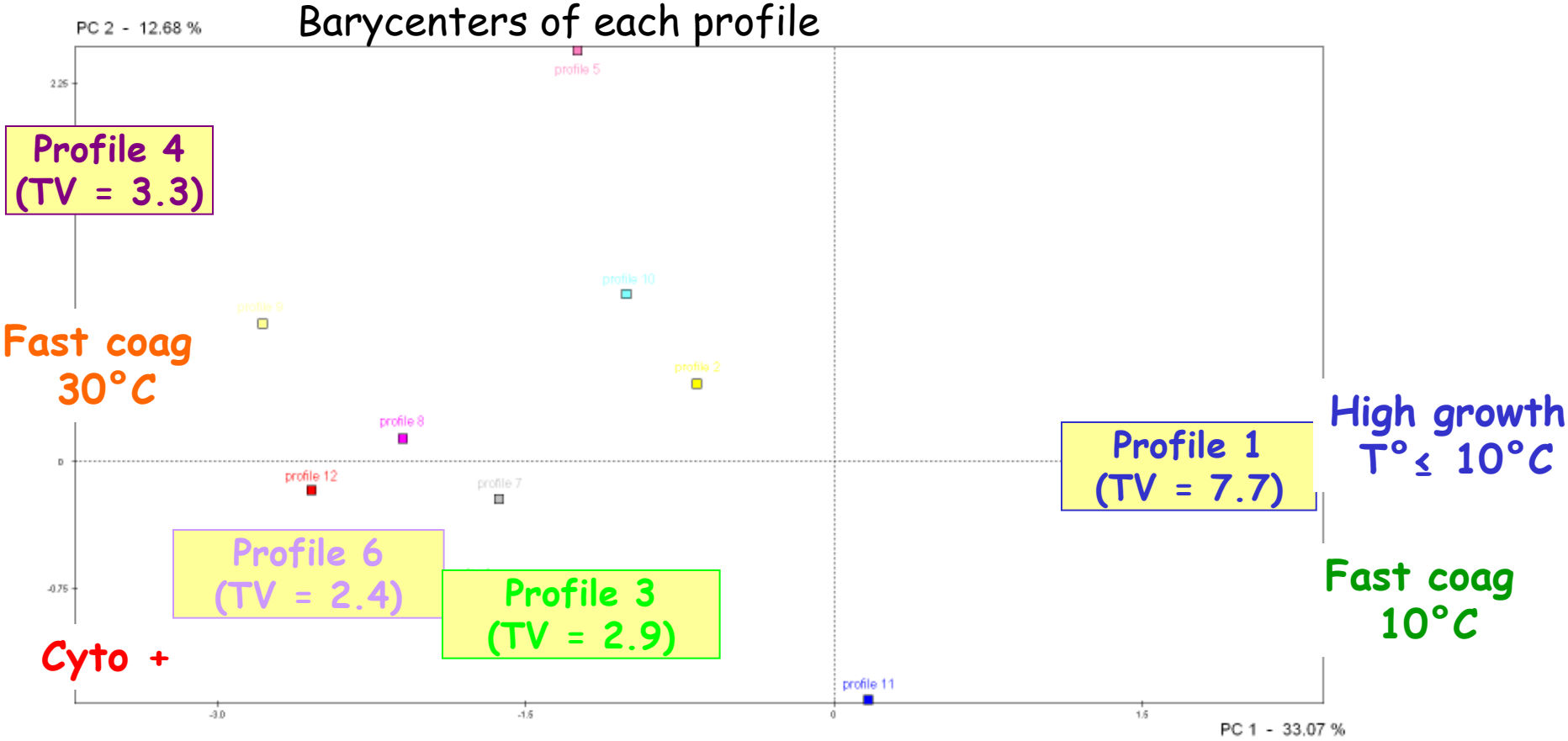
Projection of the whole collection on the plan defined by the PC 1 and 2



Global analysis of the isolates in egg product environment

- according to the profile

Barycenters of each profile

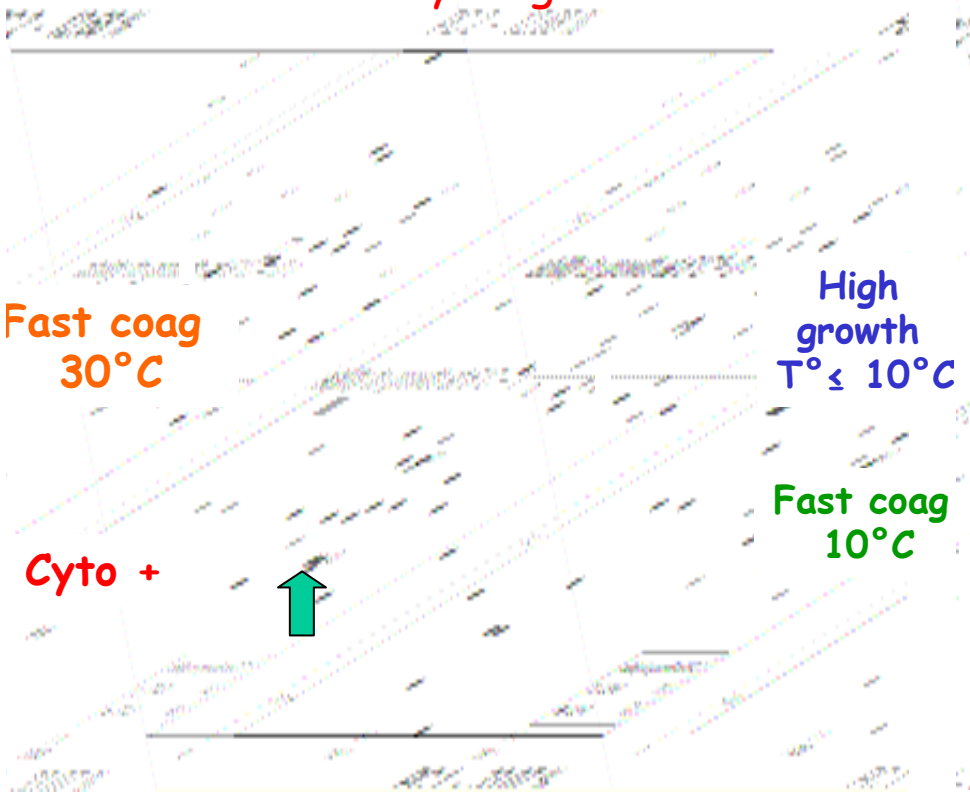


4 profiles are distinguishable

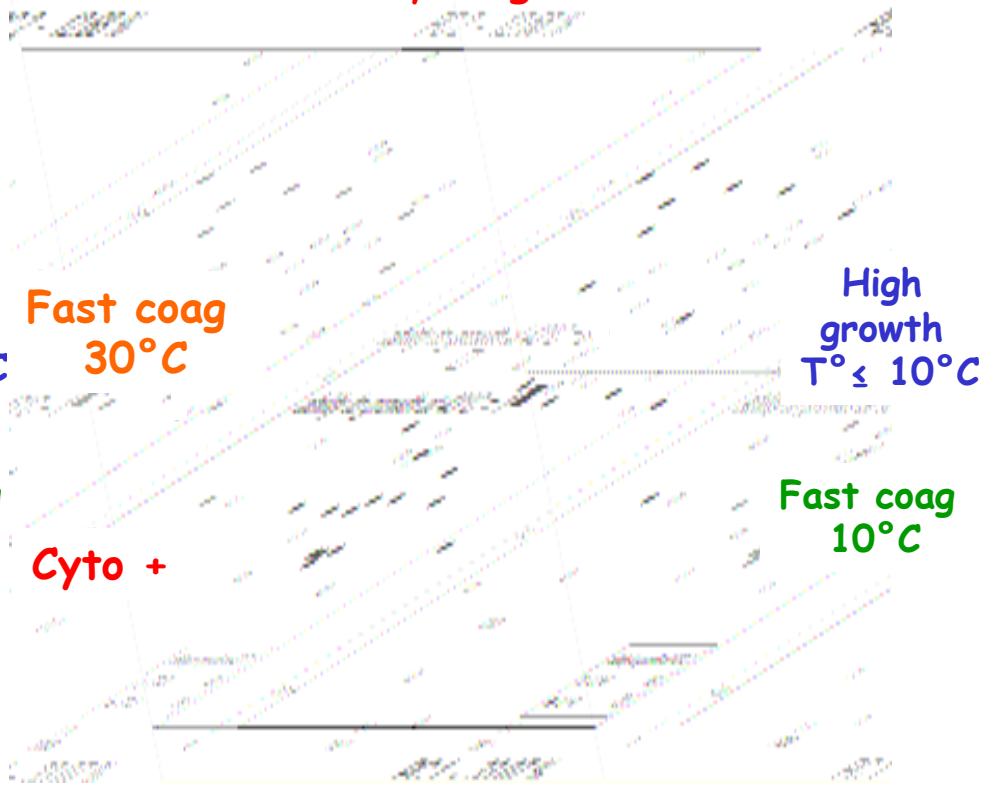
Global analysis of the isolates in egg product environment

- according to the ability to grow at 6°C or 43°C on solid medium

Projection of the whole collection
Ability to grow at 6°C



Projection of the whole collection
Ability to grow at 43°C



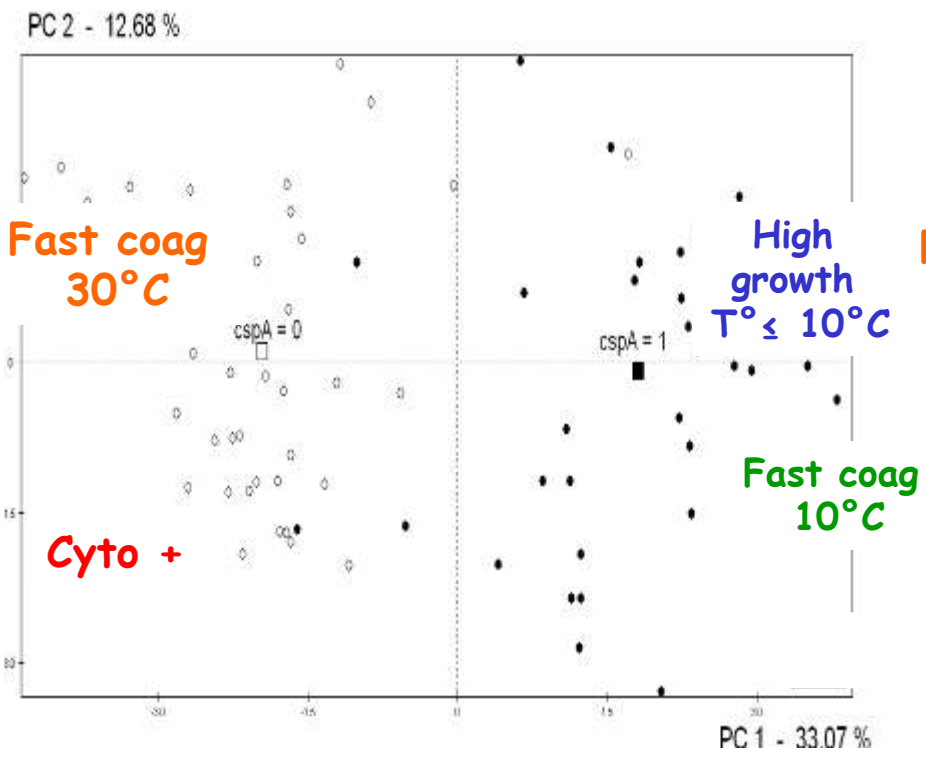
Isolates unable to grow at 6°C =
Fast egg coagulation at 30°C
(TV =4.4)
Several isolates able to grow at 6°C
are cytotoxic

Isolates able to grow at 43°C =
Fast egg coagulation at 30°C
High cytotoxicity
Isolates unable to grow at 43°C =
Opposite behaviour (TV = 6)

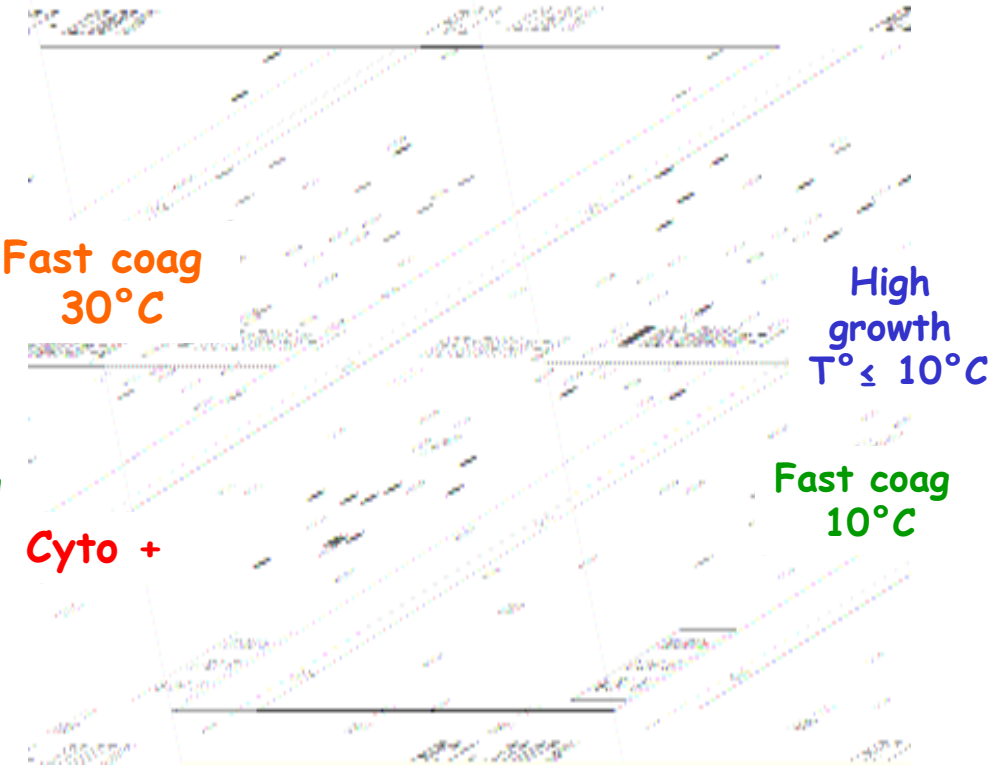
Global analysis of the isolates in egg product environment

- according to the genetic signatures

Projection of the whole collection
Presence of the *cspA* signature



Projection of the whole collection
Presence of the *16SrDNA-1m* signature



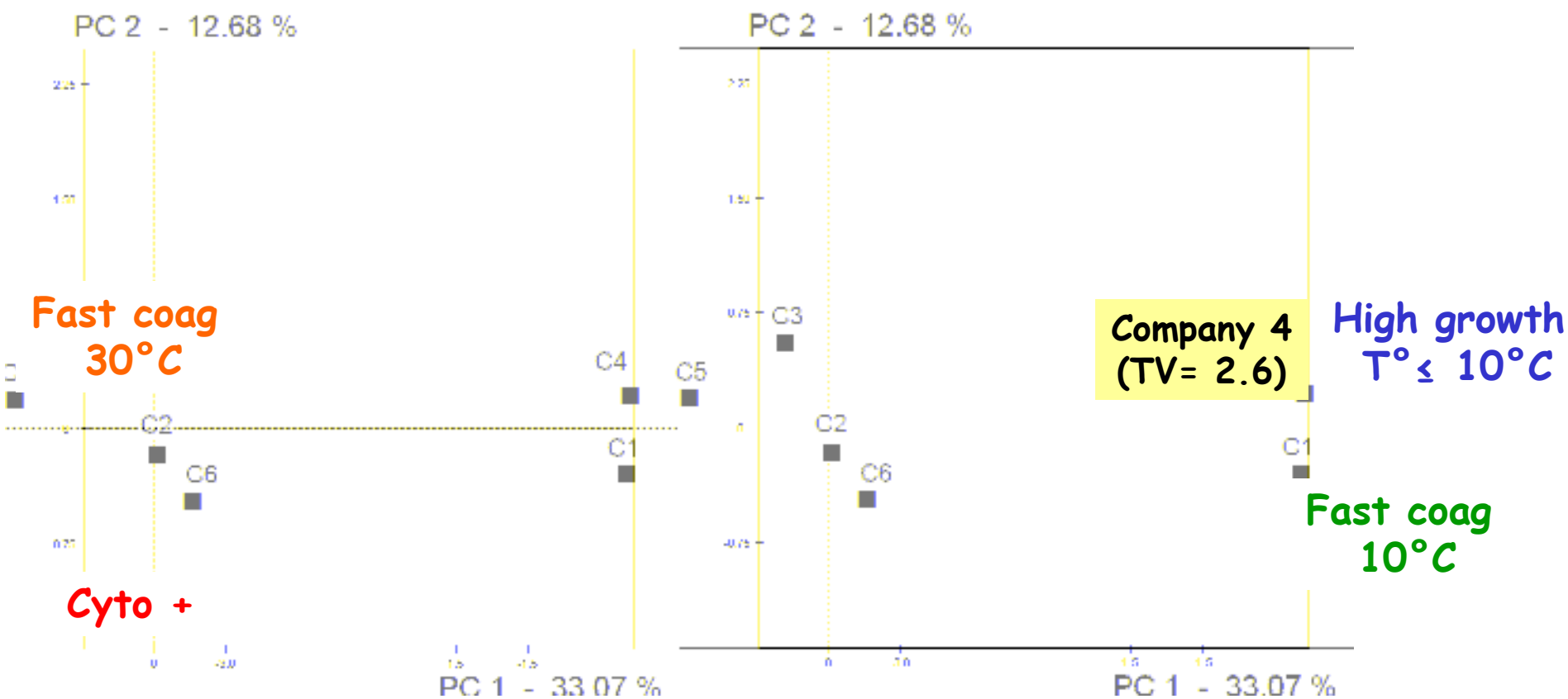
cspA signature =
Low cytotoxicity, high growth and egg coagulation at refrigerated temperature (TV= 5)

16SrDNA-1m signature =
High cytotoxicity
Fast egg coagulation at 30°C (TV= 7.2)

Global analysis of the isolates in egg product environment

- according to the origin (company)

Barycenter of each company

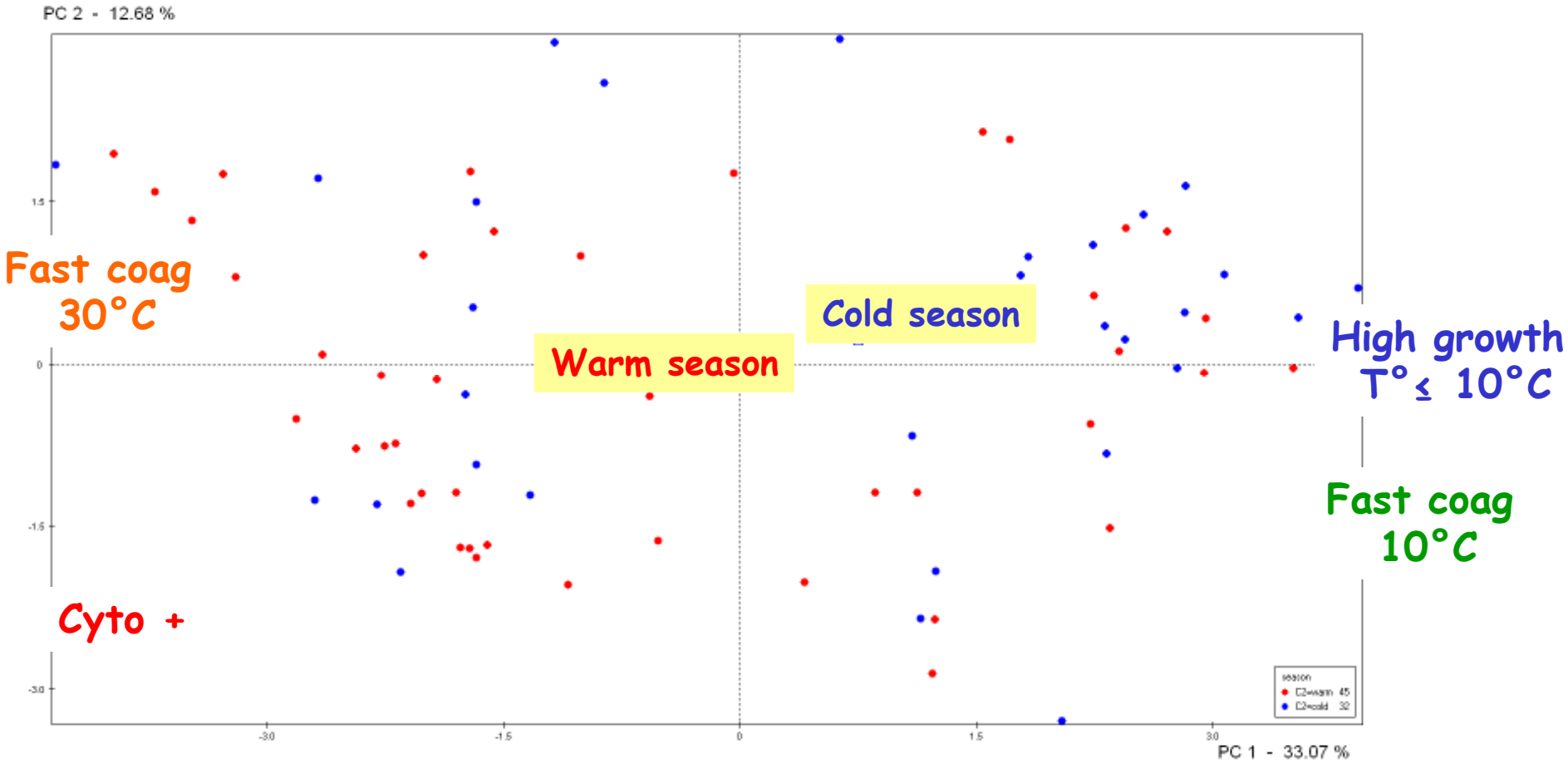


The company 4 is distinguishable

Global analysis of the isolates in egg product environment

- according to the season

Barycenter of each season



The global behavior of the isolates was slightly different at the warm and at the cold season (TV = 2.4)

Conclusions

- Liquid whole egg: optimal medium for growth and toxin production

- Main profile: profile 1 (*B. weihenstephanensis*)

  Several profiles not described in the literature: interesting behaviors

- High growth and fast egg coagulation at T°≤ 10°C correlated with:

  - presence of the *cspA* signature
  - inability to grow at 43°C

  Good markers for the spoilage risk

  ➡ **Spoilage risk**

- High cytotoxicity at 30°C and fast egg coagulation at 30°C correlated with:

  - presence of the *16SrDNA-1m* signature
  - ability to grow at 43°C

  Good markers for the health risk

  ➡ **Health risk**

New insights on psychrotrophic *B. cereus* group cells in egg product environment
➡ Starting point for their control in the food industry