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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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Florence Baron, Valérie Lechevalier-Datin, R. Belaïd, Marie-Clarisse Techer, Fabienne Gonnet, et al.. 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. IAFP European symposium, Jun 2010, Dublin, Ireland. hal-01454364

HAL Id: hal-01454364

<https://hal.science/hal-01454364>

Submitted on 3 Jun 2020

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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

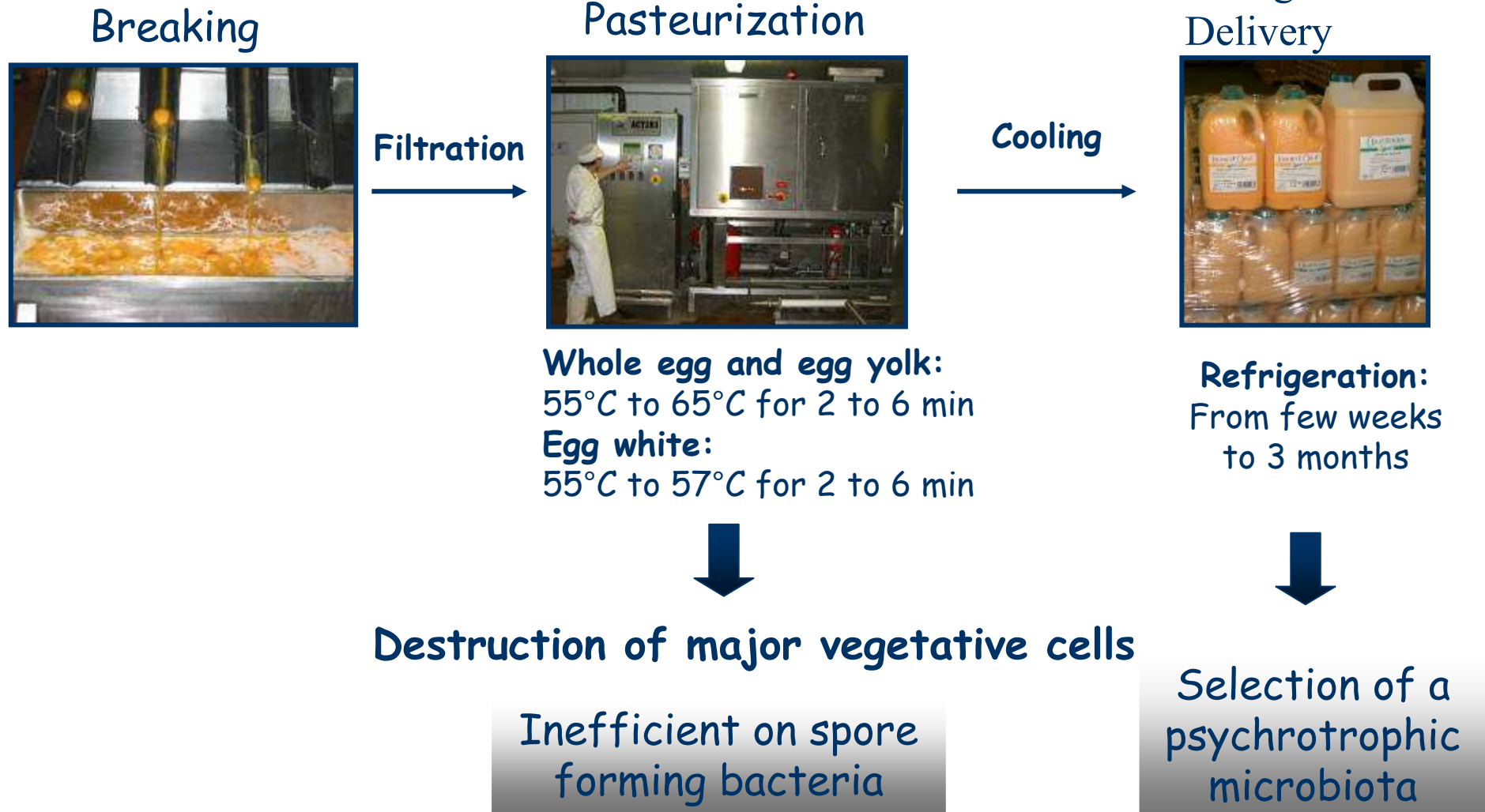
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Microbial stabilization of liquid egg products in France

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

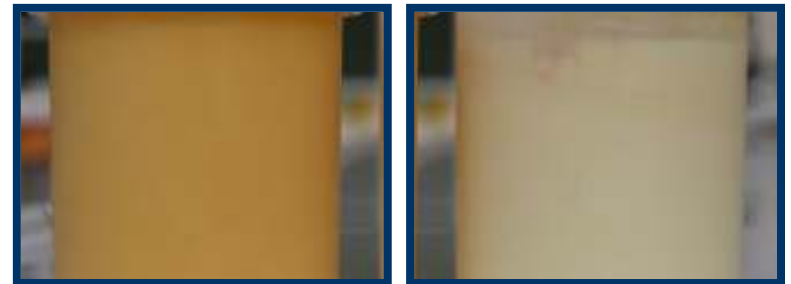


Bacteria of the *Bacillus cereus* group in liquid egg products

Properties of *B. cereus* group cells:

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

↓
Foodborne illness



Safe egg product

Spoiled egg product

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

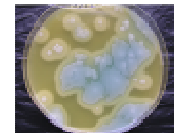


77 isolates

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	<i>cspA</i>	16S <i>rDNA-1m</i>	16S <i>rDNA-2p</i>	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) ?

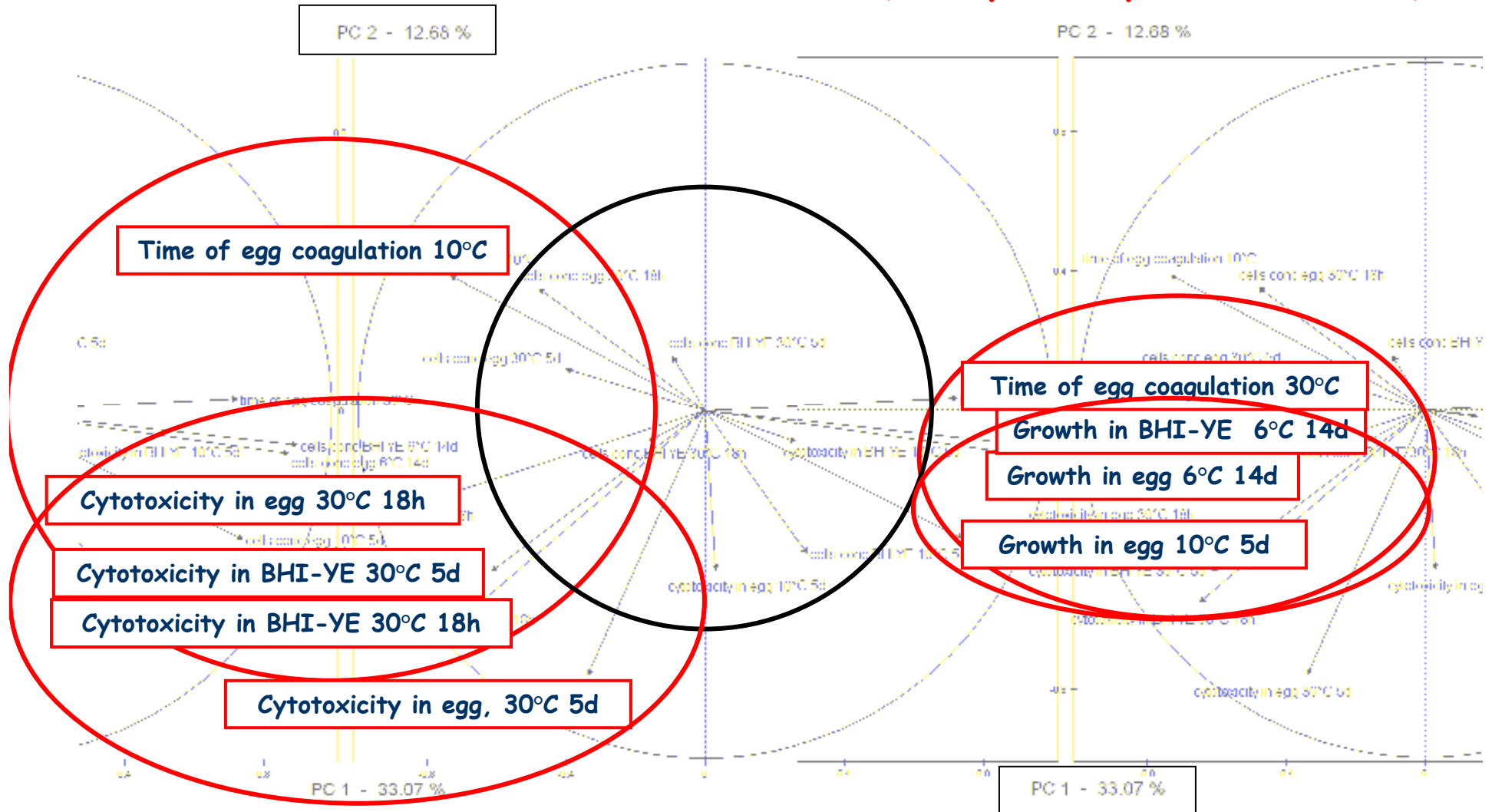
(ii) the season (warm/cold) ?



Principal Component Analysis

Global analysis of the isolates in egg product environment

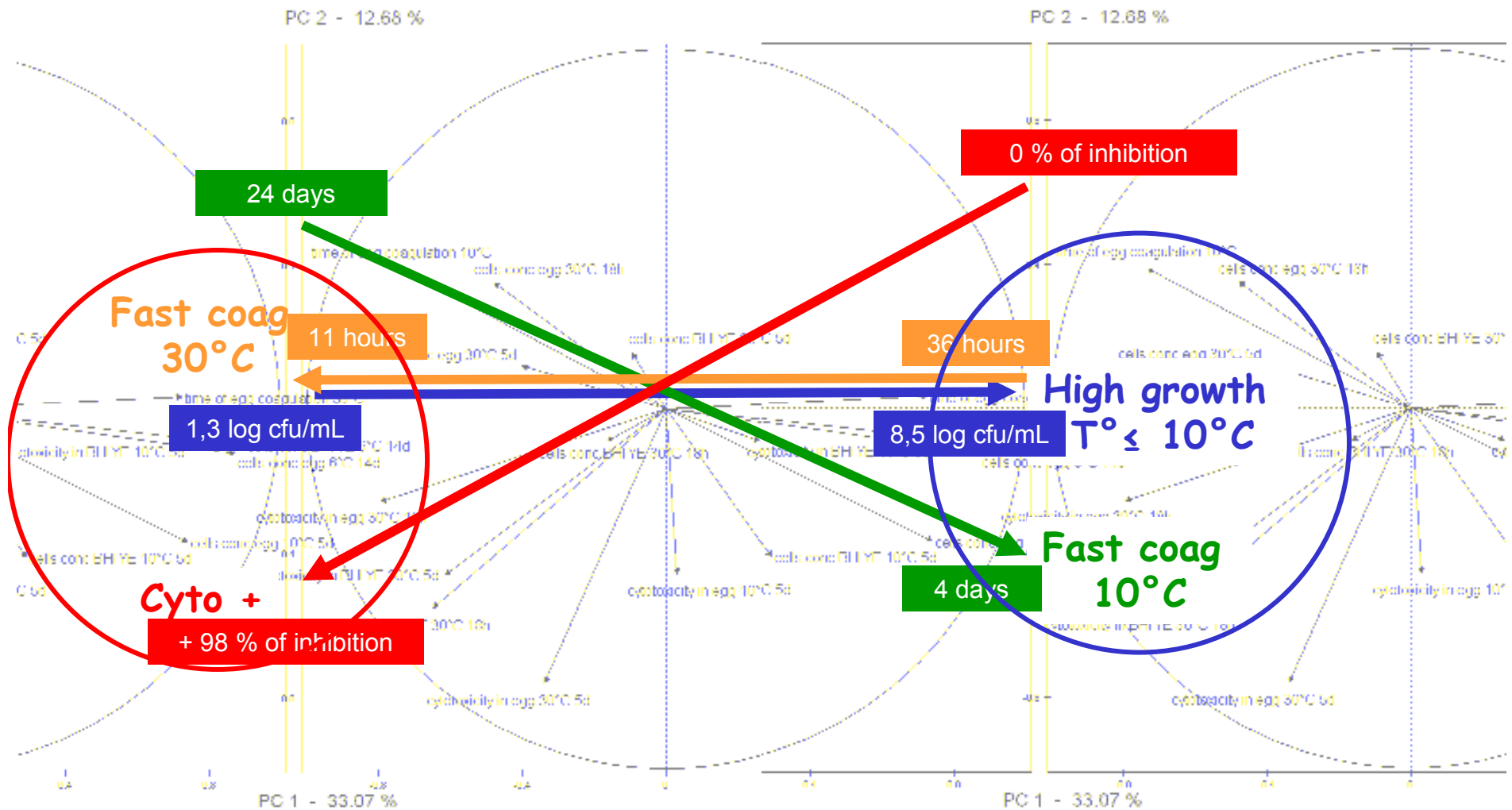
Correlation circle of the continuous variables (Principal Components 1 and 2)



- Liquid whole egg = BHI-YE for growth ($\leq 10^{\circ}\text{C}$) 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)



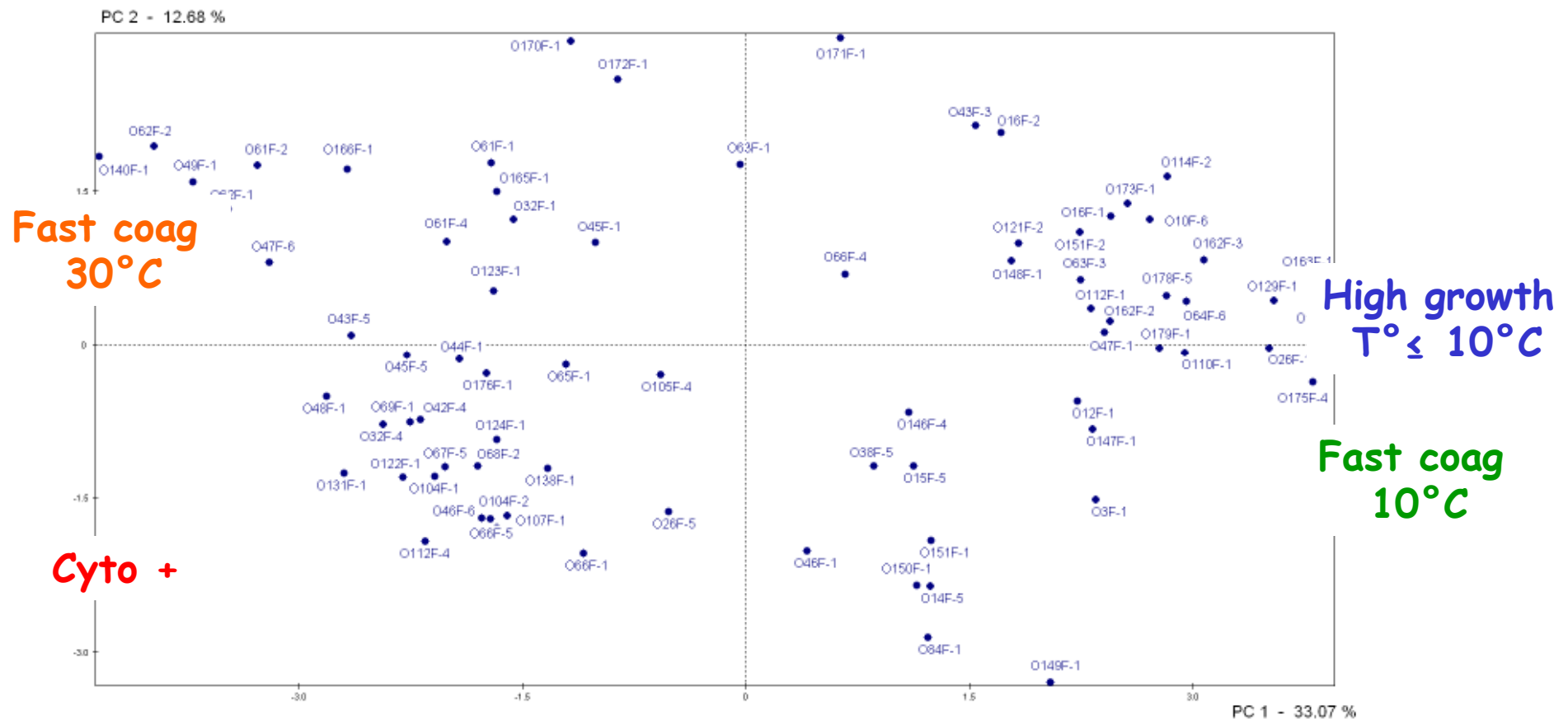
● High cytotoxic activity at 30°C
Fast egg coagulation at 30°C

≠

High growth ability at $\leq 10^\circ\text{C}$
Fast egg coagulation at 10°C

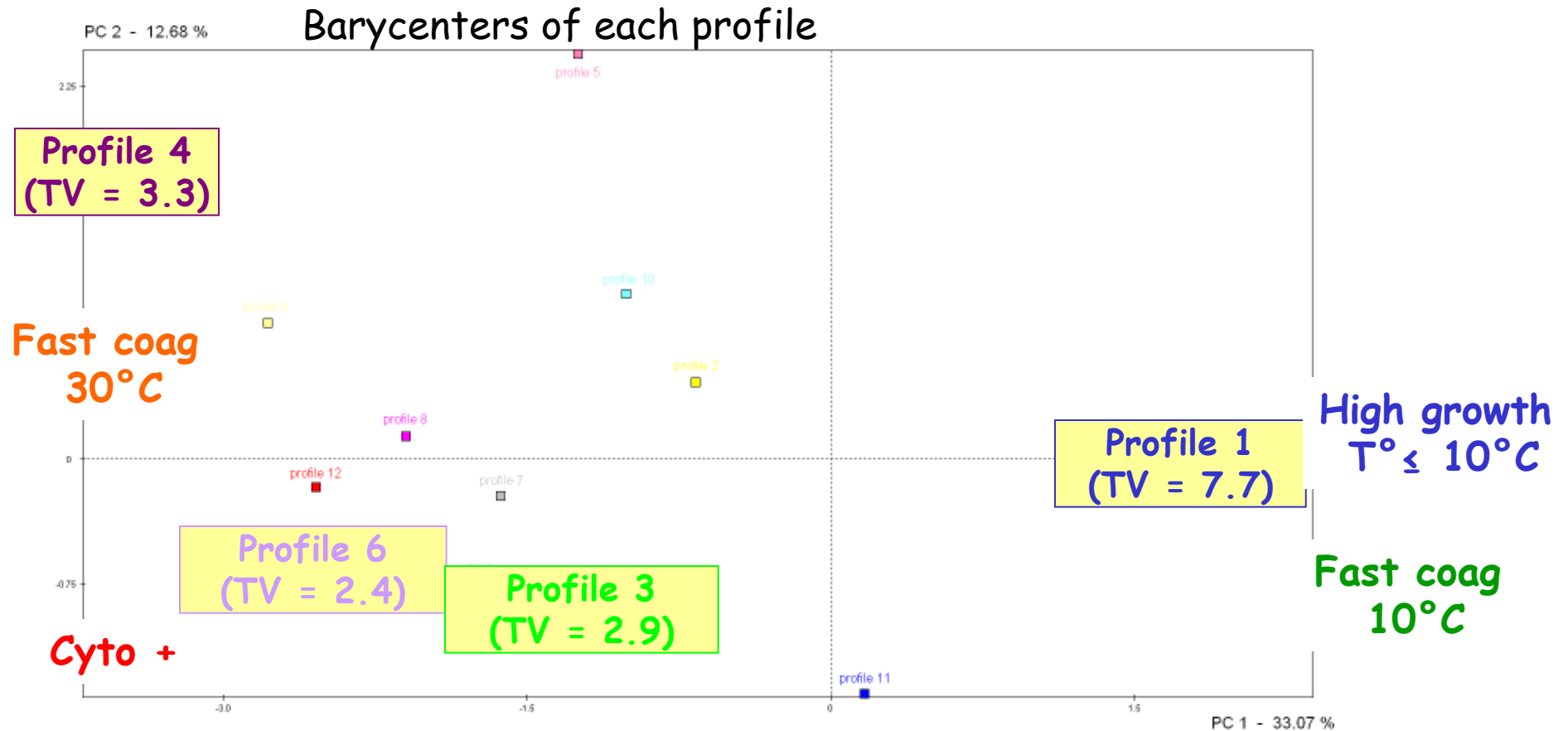
Global analysis of the isolates in egg product environment

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

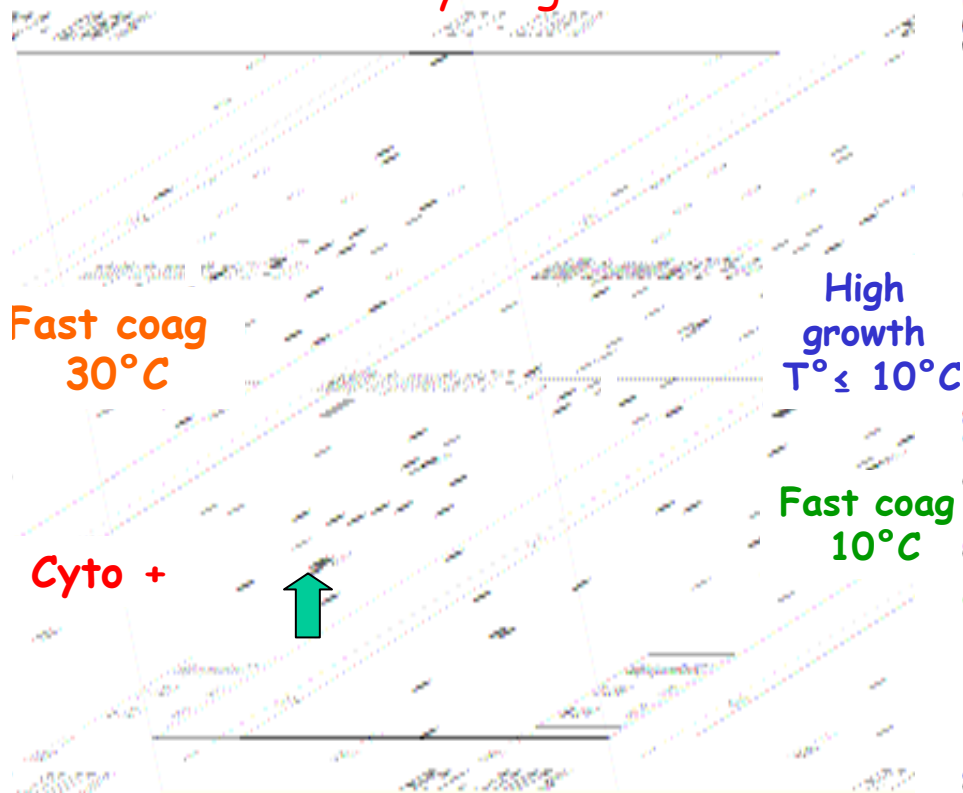


4 profiles are distinguishable

Global analysis of the isolates in egg product environment

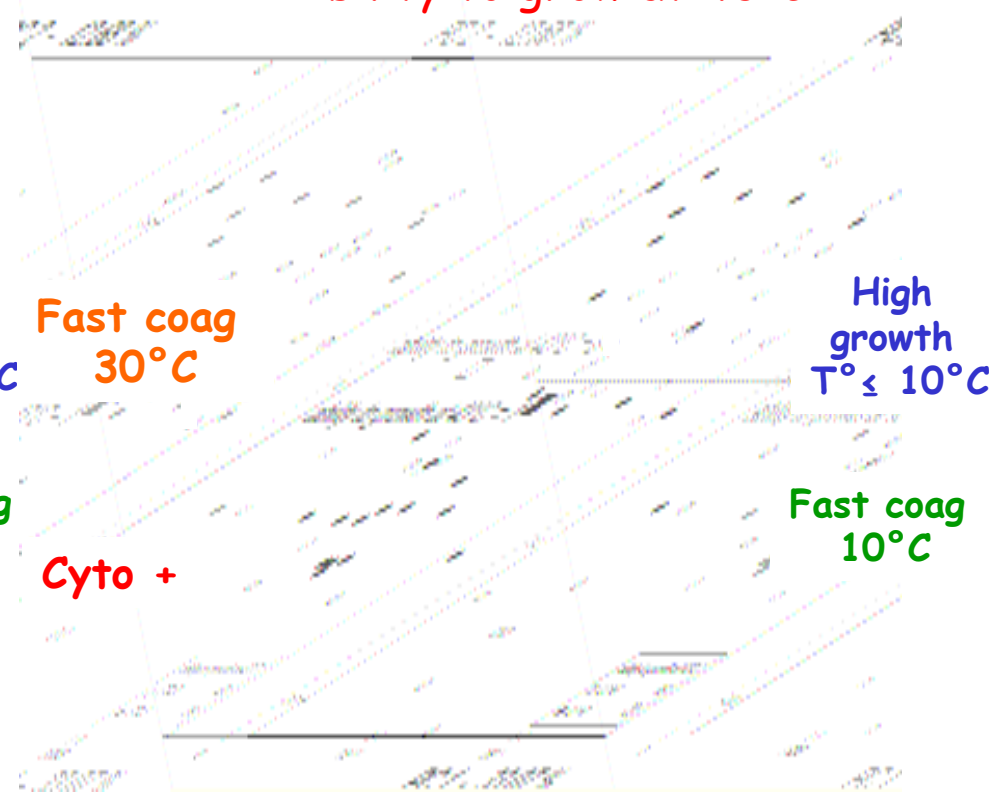
- according to the ability to grow at 6°C or 43°C on solid medium

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



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

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

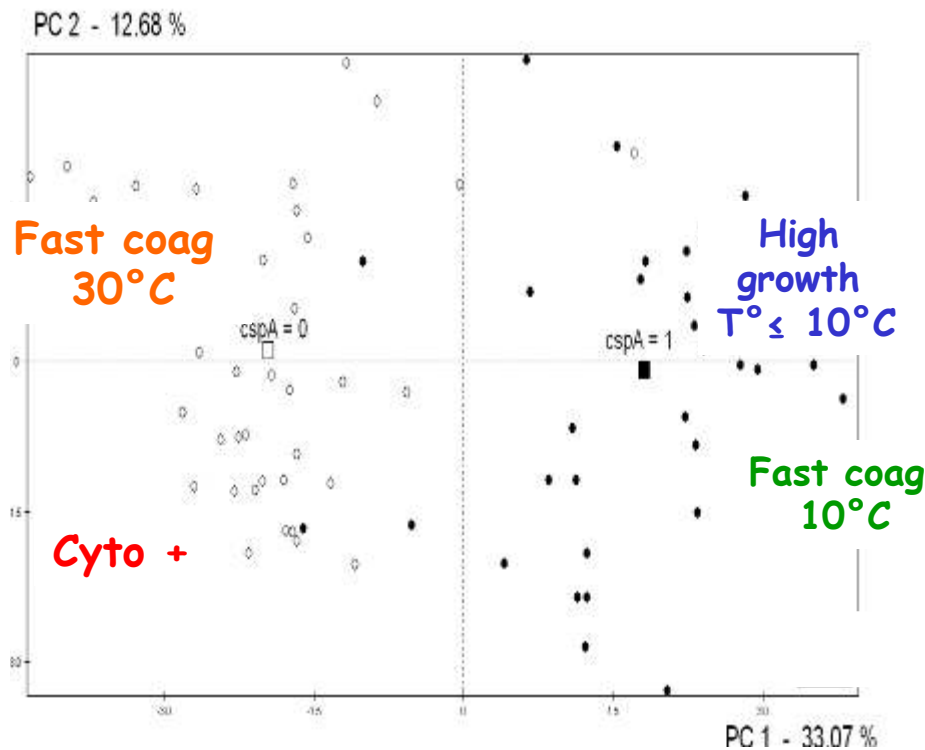


Isolates able to grow at 43°C =
Fast egg coagulation at 30°C
High cytotoxicity
Isolates unable to grow at 43°C =
Opposite behaviour (TV = 6)

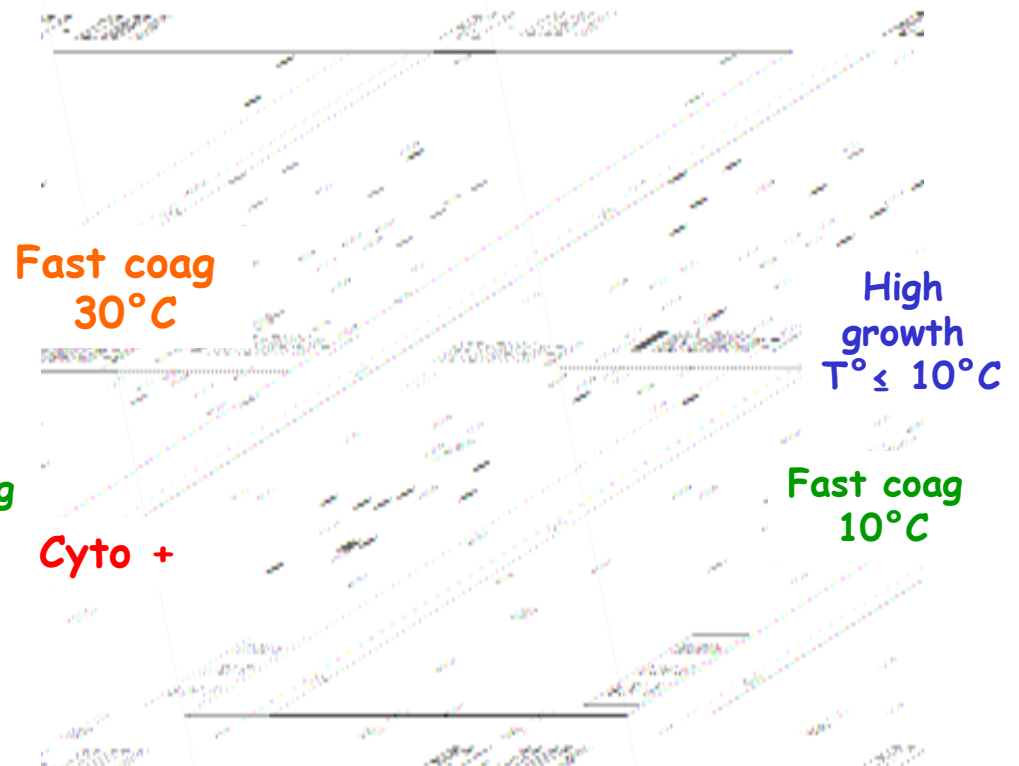
Global analysis of the isolates in egg product environment

- 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



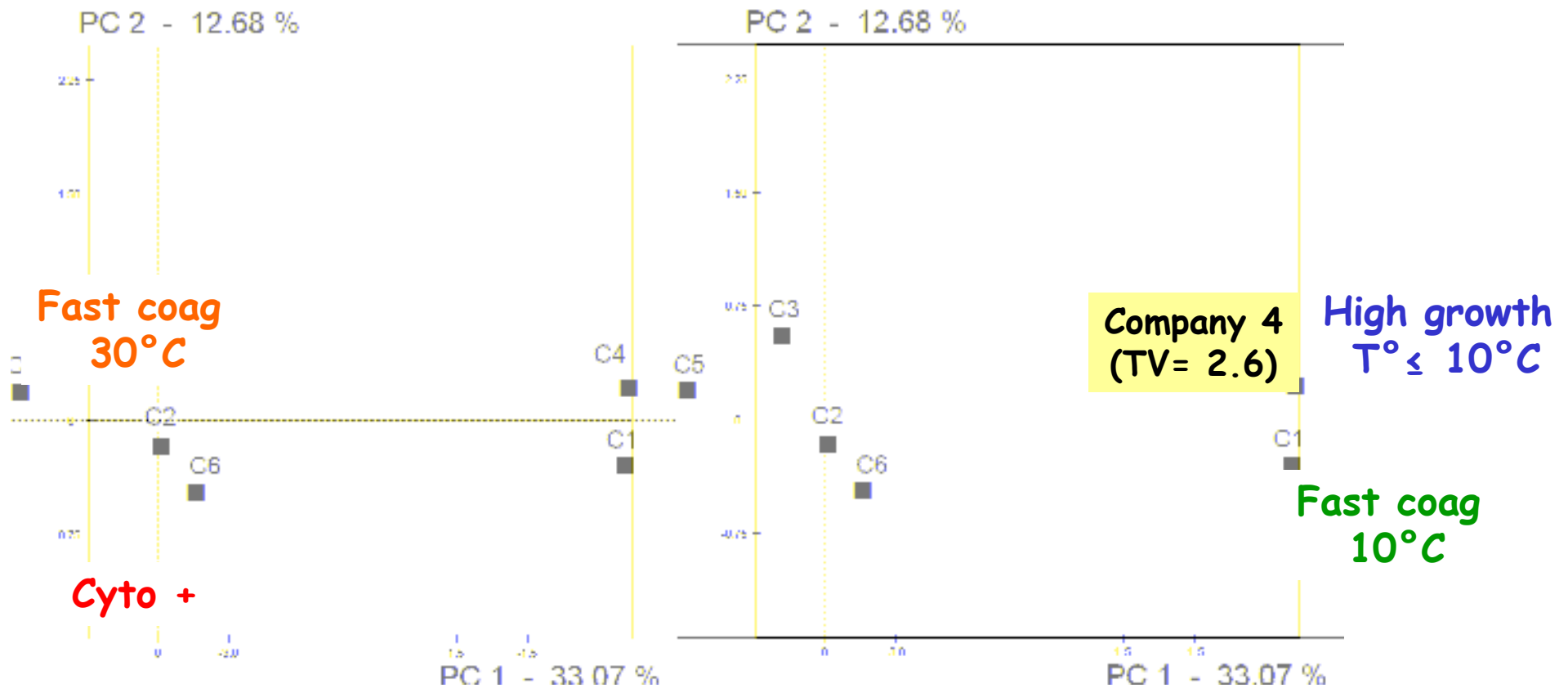
cspA signature =
Low cytotoxicity, high growth and egg
coagulation at refrigerated temperature
(TV= 5)

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

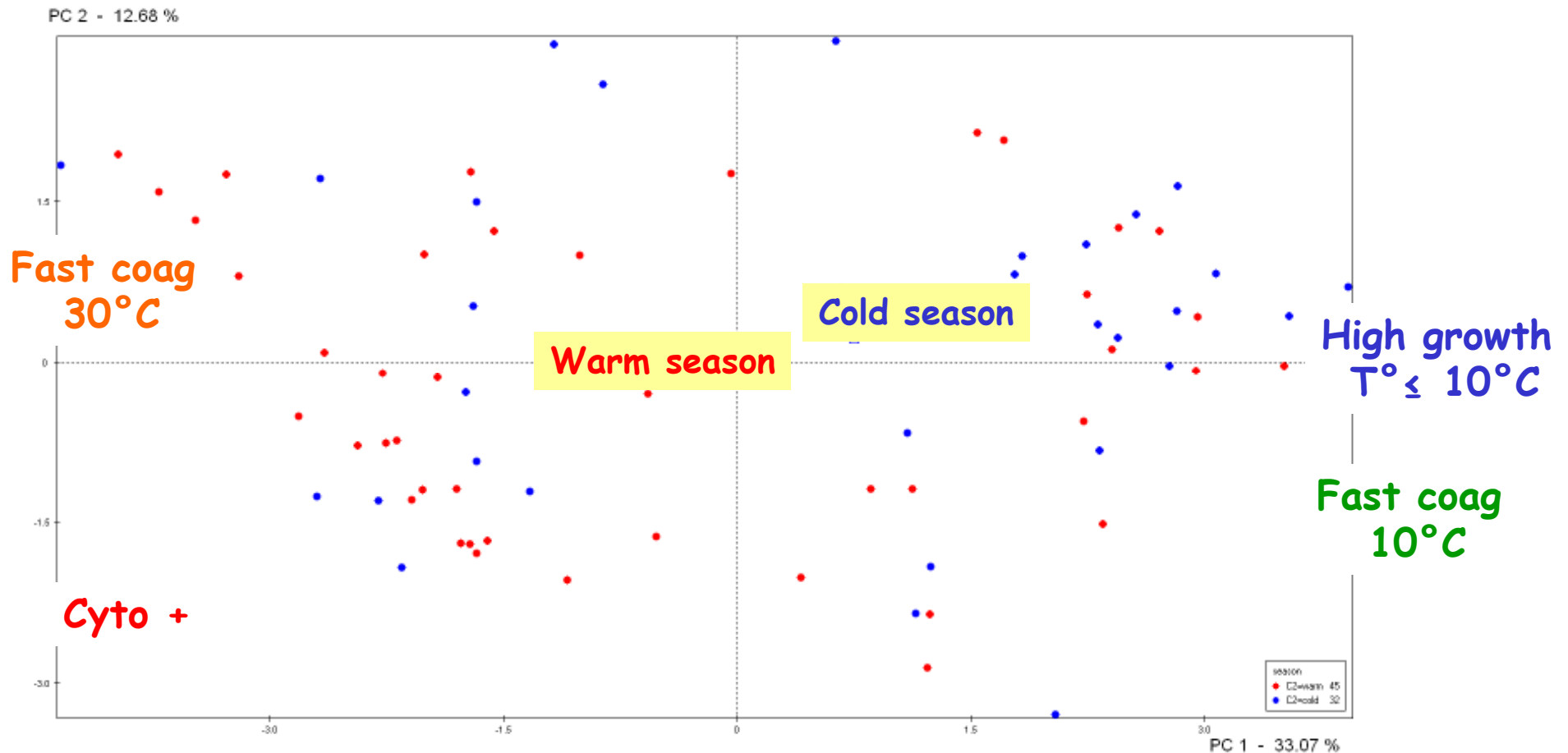


The company 4 is distinguishable

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 $T^{\circ} \leq 10^{\circ}C$ correlated with:

- presence of the *cspA* signature
- inability to grow at $43^{\circ}C$

Good markers for the spoilage risk

➡ Spoilage risk

- High cytotoxicity at $30^{\circ}C$ and fast egg coagulation at $30^{\circ}C$ correlated with:

- presence of the *16SrDNA-1m* signature
- ability to grow at $43^{\circ}C$

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