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Cheese Model to Assess the Production of Biogenic Amines by Coagulase Negative Staphylococci in Paste

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Introduction

Coagulase Negative Staphylococci (CNS) are naturally present in the environment and in some fermented foods. They are also used as starters in dairy fermentation processes because of their aromatic, enzymatic and pigmentary capacities. Most species of CNS are generally regarded as safe but their innocuity in food processing needs to be determined. In particular, the ability to produce undesirable biogenic amines (BA) in cheese matrices needs to be easily evaluated for the CNS strains which possess decarboxylase-coding genes and exhibit *in vitro* BA-production. Therefore we developed a cheese model which presents the most favorable conditions to highlight this activity. .

Material and methods

Semi-hard cheese process:

- Performed in the pilot plant of INRA-URTAL, Poligny
- Pasteurized (74°C, 30sec) milk
- Starters (see experimental design)
- Partial lactose removal step
- Salted in saturated brine (NaCl in cheese moisture=2.5%)
- Covered with wax and ripened for 4 months at 14°C

Experimental:

We used four 12 L vats (**V**) in parallel (6 min-cheeses/vat)

V1 : Two starters were combined in either high/low or low/high levels to give a low (L) or high level of proteolysis (H) respectively:

- EzaIMA400 (lactococci and *Streptococcus thermophilus*): 0.12 or 0.08 UI
- Thermophilic proteolytic lactobacilli (*Lb. helveticus* FTL 122 + *Lb. delbrueckii F 9.2.3* (1/1)): 0.2 or 1%

V2 : Starters + Control for the decarboxylase positive (DC^+) activity : *Lb. buchneri* (HistidineDC$^+$) or *Lb. brevis* (TyrosineDC$^+$) (5 Log)

V3 and V4 : Starters + one CNS strain to be tested (6 Log) :

- *S. succinus* S58B (DC^-)
- *S. capitis* 59083 (HDC^+; histamine produced *in vitro* = 248 µg/mL)
- *S. capitis* 33830 (HDC^+; histamine *in vitro*=169 µg/mL)
- *S. capitis* 54247 (TyrDC $^+$ and PheDC$^+$; tyramine *in vitro* = 15 µg/mL and ß-phenylethylamine= 22 µg/mL).

Analyses: Free amino acids index (cadmium–ninhydrine; Folkertsma et Fox, 1992 *J. Dairy Res.* 59 (2): 217-224), profile of biogenic amines (dansyl derivates; modification of Bütikofer et al., 1990 *Mitteil. Gebiete der Lebensmittel. und Hygiene* 8: 120-133), CNS (MSA 30°C, 5 d, aerobiose)

Results

1. CNS counts were maintained along the ripening period independently from the level of inoculation or composition of starters (fig. 1)
2. The highest proteolytic starter combination was elected because it produced:
   - a higher amount of precursors (free amino acids) (fig. 2)
   - a higher production of biogenic amines by the control DC^+ lactobacilli (fig. 3)
3. Biogenic amines were produced by DC^+ CNS when added, in higher amounts than with starter on its own (fig 4; see *S. capitis* 59083 and 33830) except when the *in vitro* capacity was low as for *S. capitis* 54247 which production in paste could not be differentiated from the basal level of the DC^- strain (*S. warneri* 51).
4. A basal amount of each biogenic amine ("noise") was detected in all the cheeses

Conclusions. The low-scale cheese model proposed here was able to highlight the production of biogenic amines in cheese paste when *in vitro* DC^+ CNS strains were introduced at a starter level of inoculation. The model was also able to test the production of biogenic amines by added mesophilic lactobacilli. Following this preliminary work, other *in vitro* DC^+ staphylococcus species and strains belonging to the 102 strains collection of the SCN-BEER program (1) will be tested with this model. The significant strains will be then evaluated with 3 different normal–scale non-experimental making processes to evaluate the biogenic amine formation risk by CNS in different cheese types.

(1) SCN-BEER «Coagulase Negative Staphylococci in cheeses, dry sausages, and processing plants: biodiversity and risk assessment» is funded by the French "Agence Nationale de la Recherche" (ANR-05-PNRA-005 2006-2008)

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Figure 1 : Staphylococci as influenced by the low (L) or high (H) proteolytic activity of the starters and level of inoculation of CSN (5 or 6 Log)

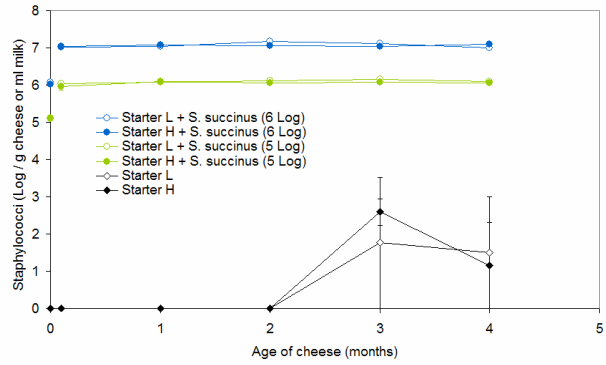

Figure 2 : Free amino acids as influenced by the low (L) or high (H) proteolytic activity of starters and level of inoculation of SCN (5 or 6 Log)

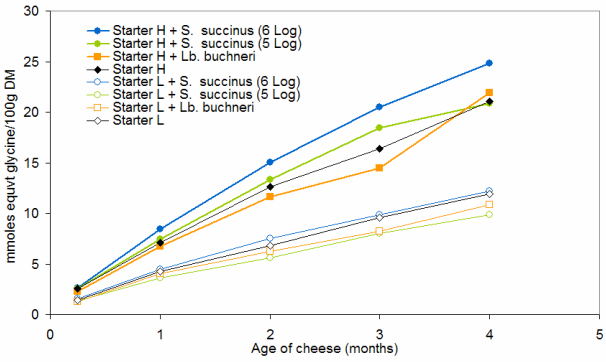

Figure 3 : Biogenic amines produced by the DC^+ control lactobacilli as influenced by the low (L) or high (H) proteolytic activity of starters

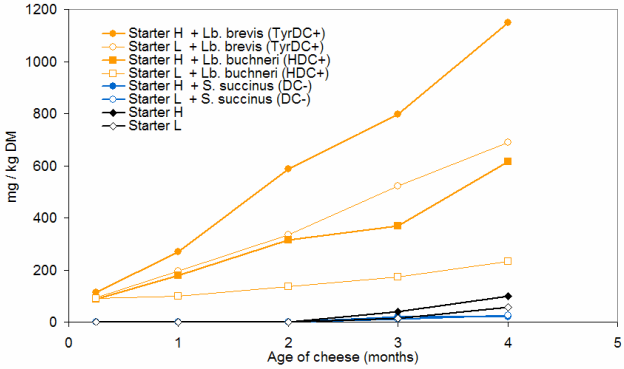

Figure 4 : Biogenic amines produced by DC+ and DC- Coagulase Negative Staphylococcus (4 months of ripening)