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Possible role of microbial interactions for growth and sporulation of *Penicillium roqueforti* in Danablu*

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Summary — Interactions between lactic acid starter cultures and *Penicillium roqueforti* used in the production of Danablu have been investigated. Based upon screening of 20 strains of *Penicillium roqueforti* and 15 strains of *Leuconostoc* ssp, *Lactobacillus* ssp *Streptococcus* ssp and *Lactococcus* ssp negative and positive interactions, ie, inhibition or stimulation of *P roqueforti* were demonstrated in laboratory media and a cheese-based model system. It was observed that the interactions were highly affected by the media used. In general positive interactions were stronger and seen more frequently in the cheese model system than in laboratory media. For *P roqueforti* as well as the lactic acid starter cultures the interactions were strain specific. The positive interactions were expressed as faster growth rate, increase of sporulation, a more intense blue/green colour and a thicker and more velvet mycelial growth. For combinations of NaCl, pH and temperatures relevant to the production of Danablu the relative importance of positive interactions increased with increasing NaCl concentrations. This applied for growth as well as sporulation. Detailed studies on the amino acid composition of the cheese based model systems inoculated with lactic acid starter cultures, *P roqueforti* or the two together indicate that the lactic acid bacteria stimulate *P roqueforti* by releasing amino acids like arginine and leucine.

interaction / *Penicillium roqueforti* / lactic acid bacteria / Danablu / amino acid

Résumé — Influence des interactions microbiennes sur la croissance et la sporulation de *Penicillium roqueforti* dans le Danablu. Les interactions entre les souches de bactéries lactiques et de *Penicillium roqueforti* utilisées dans la production de Danablu ont été étudiées. Par testage de 20 souches de *P roqueforti* et de 15 souches de *Leuconostoc*, *Lactobacillus* et *Lactococcus,* ont été mises en évidence des interactions négatives ou positives sur des milieux de laboratoire ou des milieux à base de fromage. Il a été observé que les interactions étaient fortement influencées et plus fréquentes sur les milieux à base de fromage que sur les milieux de laboratoire. S'agissant de *P roqueforti* aussi bien que des bactéries lactiques, les interactions étaient spécifiques des souches. Les interactions positives

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s'exprimaient par une vitesse de croissance plus élevée, une stimulation de la sporulation, une couleur bleue/verte plus intense, une croissance mycélienne en couche plus épaisse et plus veloutée. En ce qui concerne les différentes combinaisons de NaCl, pH et température applicables en production de Danablu, l'augmentation de l'importance relative des interactions positives a suivi l'augmentation de la concentration en NaCl. Cela était le cas pour la vitesse de croissance comme pour la sporulation. Les études de la composition en acides aminés des milieux à base de fromages ensemencés soit avec les levains lactiques, soit avec *P roqueforti,* ou avec les deux réunis ont montré que les bactéries lactiques pouvaient stimuler *P roqueforti* en produisant les acides aminés, arginine et leucine.

interaction / *Penicillium roqueforti* / bactérie lactique / Danablu / acide aminé

INTRODUCTION

As reviewed by Pederson (1979) microbial successions and interactions are considered important for a wide range of fermented foods like wine, sour dough, bread, cheese and a variety of traditional fermented products in developing countries often native to culture, countries or regions. The microorganisms responsible for the fermentations are strongly adapted to the processing technology and the ecology of the fermented product and so are the mechanisms of the microbial interactions (Colla et al, 1991; Olsen et al, 1995). Danablu cheese is a good example of the microbiological complexity of fermented foods. During ripening interactions between rennet enzymes, proteinases and lipases from milk, the lactic acid starter culture and *Penicillium roqueforti* determine maturation time, aroma, texture and appearance of the final cheese. However, the specific information available on microbial interactions in Danablu and the underlying mechanisms is very limited. Microbial interactions between cultures isolated from Roquefort cheese have been described by Devoyod and Müller (1969) and the possible role of amino acid released by lactic acid bacteria in blue veined cheeses has been reported by Gripon et al (1977). The objective of the present work was to screen several strains of *P roqueforti* isolated from blue veined cheeses and strains of lactic acid starter cultures for microbial interactions in laboratory media and a cheese based model system. Also, the study was aimed at studying the effects of NaCl at pH and temperatures relevant to Danablu production and to investigate the possible role of amino acids in stimulating growth and sporulation of *P roqueforti*.

MATERIALS AND METHODS

Cultures

P roqueforti

Twenty strains of *Penicillium roqueforti* strains used for production of Danablu and other blue veined cheeses were studied. Fifteen cultures marked Roq 1, Roq 2, Roq 4, Roq 5, Roq 8, Roq 11, Roq 14, Roq 15, Roq 18, Roq 19, Roq 20, Roq 21, Roq 22, Roq 24 and Roq 26 were supplied by Alfred Joergensen Laboratory A/S (Copenhagen, Denmark). Three strains marked PA, PJ and PV were supplied by Laboratory 'Visby' APS (Toender, Denmark). One culture marked CSL PV was produced by Centro Sperimentale Del Latte (Milan, Italy).

Lactic acid bacteria

Fifteen strains of lactic acid starter cultures were studied. Seven cultures: *Leuconostoc lactis* DB 1334, *Leuconostoc mesenteroides* ssp *cremoris* DB 1274, DB 1218, DB 1220, DB 1247 and DB 1263 and *Streptococcus thermophilus* 21 were supplied by Chr Hansen Laboratory A/S, Hoers-

holm, Denmark. Two cultures: *Lactobacillus delbrueckii* ssp *lactis* 662 and *Lactobacillus casei* ssp *casei* 160 were supplied by Laboratory 'Visby'. Further, the following six cultures were included: *Lactococcus lactis* ssp *lactis* LMG 7930, *Lactococcus lactis* ssp *cremoris* LMG 7932, *Lactococcus lactis* ssp *diacetylactis* LMG 7931, *Lactobacillus plantarum* DSM 20174, *Lactobacillus casei* ssp *casei* ATCC 393 and *Lactococcus lactis* ssp *lactis* ATCC 19435.

Diluent and media

Diluent

Salt peptone solution (SPO) containing 1 g peptone (Difco), 8.5 g NaCl, 0.3 g $Na_2HPO_4 + 2H_2O$, 10 mL Tween 80 (Merck) and 1000 mL demineralised water, pH 7.0.

Cheese agar

For the preparation of cheese agar 1.5 g bacto agar (Difco) in 500 mL demineralised water were autoclaved for 20 min at 121°C and mixed with 400 g 1-day-old unsalted Danablu containing 50% fat in dry matter (pH 4.7, a_w 0.99) received from a Danablu producing dairy and cooked for 30 min before use to inactivate the primary starter culture and *P roqueforti* strains present. pH was adjusted to pH 5.0 or 6.0 with 0.1 mol/L NaOH and was measured during incubation. The water activity (a_w) of the media was adjusted to a_w 0.99 (no addition of NaCl), a_w 0.98 (4% NaCl (w/v)), a_w 0.94 (7% NaCl (w/v)) and a_w 0.92 (10% NaCl (w/v)). Cheese agar for studies of growth and sporulation for a particular strain of *P roqueforti* was prepared from the same batch of Danablu.

MRS

MRS broth (Merck) and MRS agar (Merck) containing 1.5% bacto agar (Difco), pH 5.7 ± 0.1 were used as growth media for *Leuconostoc* ssp and lactobacilli.

LM 17

M 17 broth (Oxoid) and M 17 agar (Oxoid), pH 6.9 ± 0.2, added 1% (w/v) lactose. LM 17 broth and agar were used as growth media for lactococci and streptococci.

The diluent, MRS and LM 17 were autoclaved at 121°C for 20 min and stored at 4°C.

Measurement of water activity

The water activity (a_w) was measured using a Novasina Thermoconstanter TH 200, enBSK-4/CK-4 (Novasina AG, Zürich, Switzerland) at 25°C. Saturated salt solutions (a_w: 0.53, 0.73, 0.90 and 0.98) supplied by Novasina were used for calibration.

Preparation of suspensions of *P roqueforti* and lactic acid starter culture

From stocks of dry conidia of *P roqueforti* suspensions were prepared containing 10^6 conidia/mL estimated by microscopy.

Strains of *Leuconostoc* ssp were grown in MRS broth at 30°C, *Lactobacillus* ssp were grown in MRS broth at 37°C, *Streptococcus thermophilus* strains were grown in LM 17 broth at 37°C and *Lactococcus* ssp were grown in LM 17 broth at 30°C. All strains were incubated for 18–24 h.

For the screening assays, cell concentrations were estimated by optical density measurements at 620 nm and fresh broth added to obtain about 10^9 cells/mL. The final concentration was determined as cfu/mL in the corresponding agar media, incubated at the above temperatures for 5 days.

For the growth and sporulation experiments the lactic acid starter cultures were centrifuged at 5000 *g* for 10 min at refrigeration and washed twice in SPO. A final concentration of about 10^9 cfu/mL was obtained and controlled as described above.

During the period of experimental work the cultures were maintained on MRS or LM 17 agar slopes at 4°C.

Assays for screenings of microbial interactions

Spot test

One mL of *P roqueforti* spore suspension was poured into Petri dishes (90 mm) and mixed with 20 mL, 45°C melted cheese agar, MRS or LM 17

agar. When the agar had solidified four spots were made on each plate by addition of 40 µL lactic acid bacteria suspension to each spot. The plates were incubated for 7 days in upright position in the dark at 25°C. During growth the plates were inspected and interactions were recorded as visible stimulation or inhibition of growth and sporulation in or around the spot. Control spots were made by use of sterile MRS or LM 17 broth instead of the lactic acid bacteria suspensions.

Single lay assay

One mL *P roqueforti* spore suspension and 1 mL of suspension of lactic acid bacteria were mixed with 20 mL, 45°C, melted, cheese agar, MRS or LM 17 and poured into petri dishes (90 mm). The plates were incubated as described above. During incubation the plates were inspected and comparisons were made to agar plates containing *P roqueforti* alone, 1 mL of sterile MRS broth, and 1 mL of sterile LM 17 broth, respectively. Visible stimulation of growth and sporulation was recorded as positive interaction.

Growth rate studies for *P roqueforti*

One mL of lactic acid bacteria suspension were mixed with 20 mL melted, 45°C, cheese agar adjusted to the desired levels of NaCl, a_w and pH and poured into Petri dishes. When solidified a one point inoculation of *P roqueforti* spore suspension was made on each plate. Control plates were made of cheese agar not added lactic acid bacteria. The plates were incubated for 14 days at 10 and 25°C in upright position in the dark. During incubation the diameter of *P roqueforti* was measured. Average values from triplicate determinations are reported.

Sporulation studies for *P roqueforti*

One mL *P roqueforti* spore suspension and 1 mL lactic acid bacteria suspension were mixed with 20 mL melted, 45°C, cheese agar adjusted to the desired levels of NaCl, a_w and pH and poured into Petri dishes (90 mm). The plates were incubated at 25°C for 5 days and at 10°C for 9 days in upright position in the dark. Control plates were made with *P roqueforti* alone.

For counting of conidia the contents of one petri dish were carefully transferred to a stomacher bag, 50 mL SPO added and homogenised in a Stomacher 400 (Struers Kebo-Lab, Copenhagen, Denmark) for 2 min at high speed. For appropriate dilutions in SPO, the conidia were counted in a counting chamber (Zeiss, Oberkochen, Germany) at 400 × magnification using an Axioskop microscope (Zeiss). The examinations were carried out in duplicate. Average values are reported.

Amino acid analysis

Samples of substrates were homogenised by use of the stomacher as described above. The fat was removed from the samples by heating at 60°C for 30 min followed by cooling at 2°C for 2 h. Several filtrations were carried out with a final 0.45 µm membrane (Millipore, Molsheim, France) filtration. The filtrate was evaporated to dryness in a gentle flow of compressed air and kept frozen at –40°C until analysis. Before analysis the samples were dissolved in 5 mL 10 mmol/L HCl. The amino acid analysis was carried out according to Barkholt and Jensen (1989). The amounts of free amino acids are expressed as relative values (percentage of the total concentration of free amino acids).

RESULTS

The results from the screening of interactions between 15 primary lactic acid starter cultures and 20 strains of *P roqueforti* were strongly effected by the substrate used. Growth of *P roqueforti* together with lactic acid bacteria in several cases showed negative interactions in laboratory media but positive interactions in cheese agar (table I). For the cheese agar sign of enhanced proteolysis also appeared as softer texture of the cheese agar in the spots with positive interaction.

Table II shows the primary lactic acid starter cultures stimulating growth and sporulation of 20 *P roqueforti* strains. The results indicate strain variations for *P roque-*

Table I. Number of positive and negative interactions in laboratory media and cheese agar for 300 combinations of strains of *P roqueforti* and lactic acid starter cultures.

Nombre d'interactions positives et négatives dans le milieu de laboratoire et dans le milieu à base de fromage, pour 300 combinaisons de souches de P roqueforti *et de levains lactiques.*

	Positive interactions	*Negative interactions*	*No sign of interaction*
Laboratory media	22	195	83
Cheese agar	136	49	115

forti, eg, Roq 15 are not stimulated by any of the lactic acid bacteria, whereas six of the *P roqueforti* strains (Roq 8, Roq 11, Roq 14, Roq 20, CSL PV and PV) are stimulated by 10–12 of the lactic acid bacteria included in the screening assays. Table II also indicates strain specific variation among the lactic acid bacteria. As examples *Leuconostoc mesenteroides* ssp *cremoris* DB 1220 stimulated sixteen of the 20 *P roqueforti* (Roq 1, Roq 2, Roq 4, Roq 8, Roq 11, Roq 14, Roq 18, Roq 20, Roq 22, Roq 23, Roq 24, Roq 26, CSL PV, PA PJ and PV) strains examined whereas *Lactobacillus delbrueckii* ssp *lactis* 662 stimulated only one strain of *P roqueforti* (Roq 5). Six other strains (*Lactobacillus plantarum* DSM 20174, *Lactococcus lactis* ssp *cremoris* LMG 7932, *Lactococcus lactis* ssp *diacetylactis* LMG 7931, *Leuconostoc lactis* DB 1334, *Leuconostoc mesenteroides* ssp *cremoris* DB 1263 and *Streptococcus thermophilus* 21) stimulated between 11–13 strains of the 20 *P roqueforti* strains examined. The visible positive interactions observed in the screening assays included faster growth, stimulation of sporulation, a more intense and distinct blue-green colour and a thicker and more velvet mycelial growth of *P roqueforti.*

Table II. Strains of lactic acid starter cultures stimulating growth and sporulation of *Penicillium roqueforti* in cheese agar.

Souches de bactéries lactiques stimulant la croissance et la sporulation de P roqueforti *dans le milieu à base de fromage.*

Strains of *P roqueforti*	Strains of lactic acid bacteria stimulating *P roqueforti* [a]
Roq 1	1, 5, 8, 11, 13, 15
Roq 2	4, 5, 7, 9, 10, 11, 15
Roq 4	2, 4, 5, 9, 10, 11, 13, 15,
Roq 5	1, 3, 4, 6
Roq 8	5, 6, 7, 8, 9, 11, 12, 13, 14, 15
Roq 11	2, 5, 6, 7, 8, 9, 11, 13, 14, 15
Roq 14	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15
Roq 15	ND[b]
Roq 18	4, 7, 9, 11
Roq 19	2
Roq 20	4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15
Roq 21	2
Roq 22	8, 11
Roq 23	5, 9, 11, 13, 15
Roq 24	4, 5, 7, 8, 9, 11, 13, 14, 15
Roq 26	4, 5, 9, 11
CSL PV	2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15
PA	4, 7, 8, 9, 10, 11, 13, 14, 15
PJ	1, 5, 7, 8, 10, 11, 13, 14, 15
PV	2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15

[a] Strains of lactic acid bacteria / *Souches de bactéries lactiques*: 1) *Lactobacillus casei* ssp *casei* 160; 2) *Lactobacillus casei* ssp *casei* ATCC 393; 3) *Lactobacillus delbrueckii* ssp *lactis* 662; 4) *Lactobacillus plantarum* DSM 20174; 5) *Lactococcus lactis* ssp *cremoris* LMG 7932; 6) *Lactococcus lactis* ssp *diacetylactis* LMG 7931; 7) *Lactococcus lactis* ssp *lactis* ATCC 19435; 8) *Lactococcus lactis* ssp *lactis* LMG 7930; 9) *Leuconotoc lactis* DB 1334 10) *Leuconostoc mesenteroides* ssp *cremoris* DB 1218; *11) Leuconostoc mesenteroides* ssp *cremoris* DB 1220; 12) *Leuconostoc mesenteroides* ssp *cremoris* DB 1247; 13) *Leuconostoc mesenteroides* ssp *cremoris* DB 1263; 14) *Leuconostoc mesenteroides* ssp *cremoris* DB 1274; *15) Streptococcus thermophilus* no 21.

[b] Positive interaction not detected / *Interaction positive non détectée.*

For studies on the effect of NaCl and a_w at 10°C and 25°C on the interactions between *P roqueforti* and primary starter cultures, the strains *P roqueforti* (Roq 2) and *S thermophilus* 21 and *Leuconostoc mesenteroides* ssp *cremoris* DB 1220 were selected because of their actual use in Danablu production and demonstration of pronounced positive interactions in the screening assays. As shown in figure 1, stimulation of *P roqueforti* (Roq 2) by the lactic acid starter culture was influenced by the concentration of NaCl. Stimulation by *S thermophilus* and *L mesenteroides* ssp *cremoris*

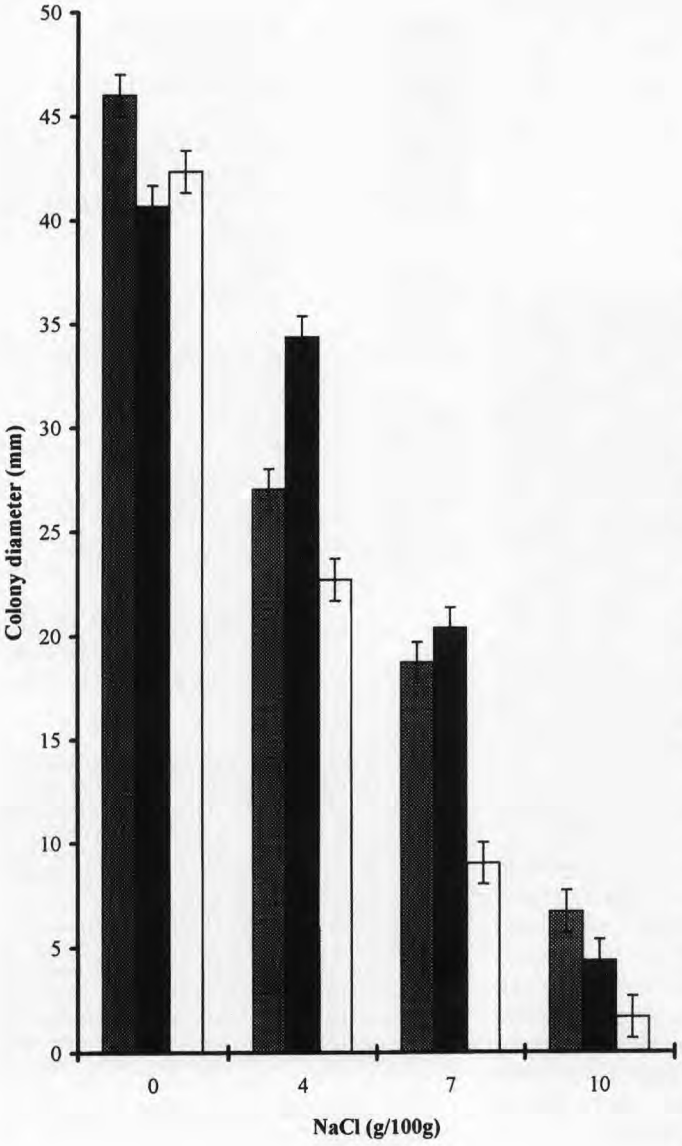

Fig 1. Colony diameter at various NaCl concentrations, pH 5.0, 25°C and 5 days of incubation. ■, Cheese agar added *Streptococcus thermophilus* 21 and *Penicillium roqueforti* (Roq 2). ■, Cheese agar added *Leuconostoc mesenteroides* subsp *cremoris* DB 1220 and *Penicillium roqueforti* (Roq 2). □, Cheese agar added *Penicillium roqueforti* (Roq 2). Standard deviation, are shown ($n = 3$) at the figure.

Diamètre de la colonie à différentes concentrations en NaCl, pH 5,0, 25 °C et 5 jours d'incubation. ■ *Milieu à base de fromage additionné de* Streptococcus thermophilus *21 et de* Penicillium roqueforti *(Roq 2).* ■ *Milieu à base de fromage additionné de* Leuconostoc mesenteroides *ssp* cremoris *DB 1220 et* Penicillium roqueforti *(Roq 2).* □ *Milieu à base de fromage additionné de* Penicillium roqueforti *(Roq 2). L'écart type est indiqué* (n = *3) sur la figure.*

was observed in the complete range of 0 to 10% NaCl (w/v), corresponding to a_w 0.99–0.92 investigated at pH 5 at 25°C in cheese agar. A relative increase of stimulation with increasing NaCl concentration was observed. At 7% NaCl (w/v) or a_w 0.94 growth stimulation by both lactic acid starter cultures corresponded to more than two-fold increase of the growth rate. At a_w 0.92, 10% NaCl (w/v) the growth rate was increased two to three-fold when *P roqueforti* grew together with *L mesenteroides* ssp *cremoris* and more than three-fold when *P roqueforti* grew with *S thermophilus*. Similar results were observed for incubation at 10°C and pH 6.0, however, at pH 6.0 the positive interactions were weaker than at pH 5.0 (results not shown).

The stimulation of sporulation by lactic acid bacteria is illustrated in figure 2. The effect is pronounced, and in the presence of 7 and 10% NaCl (w/v) a_w 0.94 and 0.92 respectively, sporulation on cheese agar at pH 5 was only observed after 9 days at 10 °C when *P roqueforti* was grown together with *S thermophilus* or *L mesenteroides* ssp *cremoris*. The lactic acid bacteria also influenced the appearance of the conidia making the *P roqueforti* growth more distinct and intense green-blue in colour. Similar results were observed for incubation at 10°C and pH 6 (results not shown).

Results from the analyses of free amino acids carried out during three consecutive days of incubation at 25°C are shown in table III for cheese agar, cheese agar inoculated with *S thermophilus* (10^9 cfu/mL), *L mesenteroides* ssp *cremoris* (10^9 cfu/mL) and *P roqueforti* (10^6 conidia/mL) respectively, and for *P roqueforti* inoculated together with each of the two lactic acid bacteria. As expected the levels of amino acids were constant in the uninoculated control plates of cheese agar. The presence of lactic acid bacteria increased the concentration of alanine, arginine, glycine, isoleucine, leucine and serine (table III) whereas the levels of aspartic acid, cysteic acid, glutamic acid, histidine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine and valine remained unchanged (results not shown). The increase seems to be of the same order of magnitude for the two cultures except for arginine which was only increased by *L mesenteroides* ssp *cremoris*. Looking upon the amino acids produced by the lactic acid bacteria and consumed by *P roqueforti* they comprise arginine and leucine when *P roqueforti* is grown together with *S thermophilus*. When grown together with *L mesenteroides* ssp *cremoris* only leucine seems to be consumed by *P roqueforti*.

DISCUSSION

The screening tests carried out in the present investigations have demonstrated that medium composition plays an important role for the interactions between *P roqueforti* and lactic acid starter cultures. The screenings also indicated that the interactions are related to the degradation of casein in the media as positive interactions were predominant in cheese agar compared with laboratory media not containing casein. They also demonstrated that interactions are strain-specific for *P roqueforti* as well as for the lactic acid bacteria. Therefore, to make use of positive interactions in practice cultures have to be selected according to such specific properties. The benefits obtainable by selecting the right combination of cultures are emphasised by the finding that the stimulation of *P roqueforti* growth as well as sporulation is relatively stronger at high levels of NaCl and low a_w values as seen in Danablu ranging from 0.2 to 8% (w/w) corresponding to a_w-values ranging from 0.99 to 0.90 during the period of *P roqueforti* growth and sporulation. Stimulation of sporulation is very important in the way that the conidia contribute to proteoly-

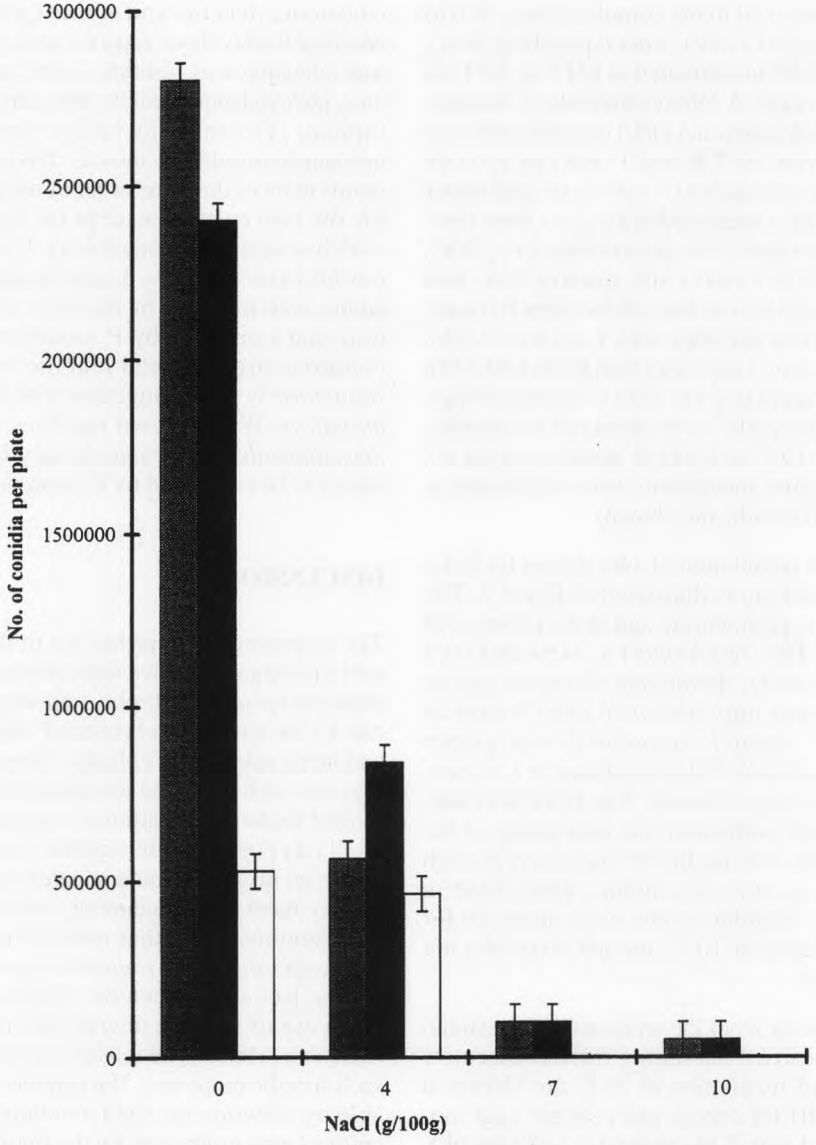

Fig 2. Number of conidia per plate at various NaCl concentrations, pH 5.0, 10°C and 9 days of incubation. ■, Cheese agar added *Streptococcus thermophilus* 21 and *Penicillium roqueforti* (Roq 2). ■, Cheese agar added *Leuconostoc mesenteroides* subsp *cremoris* DB 1220 and *Penicillium roqueforti* (Roq 2). □, Cheese agar added *Penicillium roqueforti* (Roq 2). Standard deviation, are shown ($n = 2$) at the figure.

Nombre de conidies par boîte à différentes concentrations en NaCl, pH 5,0, 10 °C et 9 jours d'incubation. ■ *Milieu à base de fromage additionné de* Streptococcus thermophilus *21 et de* Penicillium roqueforti *(Roq 2).* ■ *Milieu à base de fromage additionné de* Leuconostoc mesenteroides *ssp* cremoris *DB 1220 et* Penicillium roqueforti *(Roq 2).* □ *Milieu à base de fromage additionné de* Penicillium roqueforti *(Roq 2). L'écart type est indiqué* (n = 2) *sur la figure.*

Table III. Concentration of free amino acids after 24, 48, and 72 h of incubation at 25°C and pH 5.0 in cheese agar, cheese agar inoculated with *Streptococcus salivarius* ssp *thermophilus*, *Leuconostoc mesenteroides* ssp *cremoris* DB 1220, *Penicillium roqueforti* together with *Streptococcus salivarius* ssp *thermophilus* 21, and *Penicillium roqueforti* together with *Leuconostoc mesenteroides* ssp *cremoris* DB 1220.

Concentration en acides aminés libres après 24, 48 et 72 heures d'incubation à 25 °C et pH 5,0 dans le milieu à base de fromage, et dans ce même milieu inoculé avec Streptococcus thermophilus, Leuconostoc mesenteroides *ssp* cremoris *DB 1220*, Penicillium roqueforti + Streptococcus salivarius *ssp* thermophilus *21, et* Penicillium roqueforti + Leuconostoc mesenteroides *ssp* cremoris *DB 1220.*

Amino acids	*Incubation time (h)*	*Sheese agar*	S thermophilus	L cremoris	P roqueforti	S thermophilus + P roqueforti	L cremoris + P roqueforti
Alanine	24	5.8	ND	10.6	5.0	6.5	8.7
	48	5.8	8.6	10.9	ND	11.1	10.7
	72	5.8	9.6	12.4	5.2	11.0	8.7
Arginine	24	0.6	ND	3.9	1.3	3.7	2.2
	48	0.6	0.1	3.5	ND	0.8	4.4
	72	0.6	0.1	3.6	1.6	0.5	3.2
Glycine	24	1.8	ND	5.5	1.4	4.5	3.7
	48	1.8	4.7	4.6	ND	3.8	3.8
	72	1.8	5.3	5.4	4.4	5.3	5.4
Isoleucine	24	1.3	ND	3.2	1.3	1.9	2.0
	48	1.3	3.3	3.1	ND	2.2	1.7
	72	1.3	3.3	3.2	2.3	3.2	2.9
Leucine	24	9.9	ND	11.0	8.8	12.0	10.6
	48	9.9	13.4	11.7	ND	8.3	5.1
	72	9.9	13.3	12.4	6.3	7.6	8.5
Serine	24	0.0	ND	5.7	2.6	5.2	4.8
	48	0.0	0.0	2.4	ND	4.7	5.5
	72	0.0	2.1	4.7	7.7	9.3	11.7

L cremoris = *Leuconostoc mesenteroides* ssp *cremoris* DB 1220. *S thermophilus* = *Streptococcus thermophilus.* ND = Not determined / *non déterminé.*

sis and lipolysis (Fan et al, 1976; Chalier and Crouzet, 1993), give rise to aroma components (Fan et al, 1976) and determine the blue colour of the cheese. Apart from enhancing maturation and improving the appearance of the cheese in general, the positive interactions in particular may promote growth and sporulation in the outer part of the cheese which is the part of the Danablu with the highest concentration of NaCl and lowest a_w.

The results obtained from the amino acid analysis pointed at arginine and leucine as possible stimulating agents for *P roqueforti* growth and sporulation. According to Philipp (1981) leucine is hardly metabolised by *P roqueforti*. This apparent discrepancy may be explained by the origin of the strains investigated by Philipp (1981). These isolates may in fact be very different taxonomically from the present strains (Boysen et al, 1996). The possible role of amino acids

for the positive interactions are supported by the specific effect of cheese agar with its content of casein as compared to the laboratory media investigated in the present work not containing casein. It is also supported by the results obtained from Gripon et al (1977) showing a synergistic action between *Penicillium* proteases and *S thermophilus* in the breakdown of casein, but not indicating the significance of any particular amino acids.

Further studies are needed to fully understand the mechanisms of positive interactions and large scale dairy trials should be carried out to prove the importance of the positive interactions in Danablu production.

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