ELSEVIER

International Dairy Journal 10 (2000) 263–270

INTERNATIONAL DAIRY JOURNAL

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The technological characteristics of *Debaryomyces hansenii* and *Yarrowia lipolytica* and their potential as starter cultures for production of Danablu

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Received 22 September 1999; accepted 13 May 2000

Abstract

Six strains of *Debaryomyces hansenii* var. *hansenii* and *Yarrowia lipolytica*, respectively, originating from blue mould cheeses were examined for their potential use as starter cultures for the production of Danablu in laboratory studies. *D. hansenii* showed strong growth and assimilation of lactose, galactose, lactate and five out of six strains assimilated citric acid under the environmental conditions prevailing in Danablu during maturation at 10°C. *Y. lipolytica* was more sensitive to NaCl and did not assimilate lactose and galactose. Both yeasts hydrolysed tributyrin with the highest activity observed for *Y. lipolytica*. *D. hansenii* showed little if any release of free fatty acids from butterfat at 10°C. *Y. lipolytica* was strongly lipolytic. The strains of *D. hansenii* were not able to hydrolyse casein at 10°C whereas 4 of the 6 strains of *Y. lipolytica* degraded all components of the casein. Strain-specific interactions, in cheese agar resulting in inhibition of mycelial growth and sporulation of *P. roqueforti* was observed, especially for *Y. lipolytica*. © 2000 Published by Elsevier Science Ltd.

Keywords: *Debaryomyces hansenii*; *Yarrowia lipolytica*; Danablu; Starter Cultures; Enzymatic activities; Interactions

1. Introduction

Yeasts often occur in high numbers, 10^5–10^8 cfu^{-1}g, in Danablu (van den Tempel & Jakobsen, 1998), even they are not added as a starter culture. The origin of the yeasts seems to be strains from milk surviving heat treatment and strains established in the cheese brine and other microenvironments in the dairies (van den Tempel & Jakobsen, 1998). The predominant yeasts seen in Danablu are strains of *Debaryomyces hansenii*, or its anamorph form *Candida famata. Yarrowia lipolytica* or its anamorph form *Candida lipolytica* is also found regularly in Danablu although in lower numbers (10^3 cfu^{-1}g) (van den Tempel & Jakobsen, 1998). However, the very strong lipolytic and proteolytic activity of *Y. lipolytica* (Guerzoni, Gobbetti, Lanciotii, Vannini & Chaves López, 1998) indicate that it could contribute to the maturation of Danablu if used as a starter culture, as suggested for other cheeses by Guerzoni et al. (1998). The technological properties of *D. hansenii* and *Y. lipolytica* under the conditions prevailing during maturation of Danablu or related blue mould cheeses have not been investigated.

According to Kurtzman and Fell (1998) the species *D. hansenii* has two varieties i.e. var *hansenii* and var. *fabryi*. Physiologically the two varieties only can be distinguished by maximum growth temperatures. Pronounced phenotypical differences have been found for isolates of *D. hansenii* from Danablu (van den Tempel & Jakobsen, 1998). *Y. lipolytica* is the only species of the genus *Yarrowia* and it is only distantly related to most other known ascomyceteous yeasts (Kurtzman & Fell, 1998). Isolates of *Y. lipolytica* from previous studies of Danablu were found to be phenotypically similar (van den Tempel & Jakobsen, 1998).

It has been the main objective of the present study to characterise the technological properties of different *D. hansenii* and *Y. lipolytica* under conditions of temperature, pH, levels of NaCl and water activity (a_w) typical for the ripening of Danablu. The examinations carried

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0958-6946/00/$ - see front matter © 2000 Published by Elsevier Science Ltd.
PII: S0958-6946(00)00053-4

out include growth kinetics, assimilation of lactose, galactose, lactate and citric acid, hydrolysis of tributyrin, butter fat and casein, interactions with *Penicillium roqueforti* starter cultures and pigment formation in cheese agar.

2. Materials and methods

2.1. Cultures

Six strains of *Debaryomyces hansenii* and six strains of *Yarrowia lipolytica* were studied. Four strains of *D. hansenii* (FB1, 3, 78 and 135) and five strains *of Y. lipolytica* (marked FB14, 30, 77, 132 and 138) were isolated from Danablu and identified as described by van den Tempel & Jakobsen (1998). One strain of *D. hansenii* (FB2) was isolated from Roquefort cheese and identified according to Kreger-van Rij, 1984 (unpublished data). The remaining strains were reference cultures of *D. hansenii* (CBS 164) and *Y. lipolytica* (CBS 2075) obtained from "Centraalbureau voor Schimmelcultures" (Delft, The Netherlands).

Three strains of *Penicillium roqueforti*, Roq 1, CSL and PV used for the production of Danablu were included in interaction studies with the yeasts. They were obtained from Alfred Joergensen Laboratory A/S (Copenhagen, Denmark), Centro Sperimentale Del Latte (Milan, Italy) and Laboratory "Visby" APS (Toender, Denmark), respectively. Roq 1 has weak and PV and CSL have strong proteolytic and lipolytic activities as determined by hydrolysis of casein and release of free fatty acids from butterfat emulsions (Larsen, Rotvig Kristiansen & Kronborg Hansen, 1998; Larsen & Jensen, 1999).

2.2. Growth experiments

Inocula were grown in Malt–Yeast–Peptone–Glucose Broth (MYGP), pH 6.5, containing, g L^{-1}: yeast extract (Difco, Michigan, USA), 3.0, malt extract (Difco), 3.0, bacteriological peptone (Oxoid, Bakingstoke, UK), 5.0 and glucose (Merck, Darmstadt, Germany), 10.0, for 2 days at 25°C. The inoculation size was 10^3 cells mL^{-1}. The pH and water activity (a_w) limits for growth at 10°C were determined in a laboratory medium containing, g L^{-1}: glucose (Difco), 50.0, Yeast Nitrogen Base (Difco) 67.0 and demineralised water to 1000 mL at different pH (4.0, 5.0, 5.5 and 6.0) and different a_w values (0.92, 0.94, 0. 96 and 0.99). The pH studies were carried out in the medium at a_w 0.99; the pH was adjusted with 1 M NaOH and 1 M HCl and measured with a combined pH electrode (U402-88TE-S7, Ingold, Urdorf, Switzerland) connected to a pH meter (PHM 80, Radiometer, Copenhagen, Denmark). The a_w studies were carried out at pH 5.5 and the a_w was adjusted by addition of NaCl (0, 2, 4, 6, 8, 10, 12 and 14% w/v) and measured with a Novasina Thermoconstaner 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. The growth was estimated visually using Wickerhams card (Wickerham & Burton, 1948) once a week over a period of 3 weeks. All examinations were carried out on two different occasions.

2.3. Assimilation of lactose, galactose, lactate and citric acid

Assimilations at different levels of NaCl (0, 2, 4, 6, 8, 10, 12 and 14% w/v) at pH 5.5 and 10°C were investigated according to Kurtzman and Fell (1998).

2.4. Determination of lipolytic activity

The lipolytic activity was determined by agar diffusion test on 0.1% tributyrin at 10°C as described by van den Tempel and Jakobsen (1998) and by titration of free fatty acids from butter fat emulsions as described by Sørensen and Samuelsen (1996). The emulsions (pH 5.5, 0 and 4% (w/v) NaCl) were incubated for 10 days at 10°C. At the end of incubation they were checked for purity by streaking onto Potato Dextrose Agar (PDA, Merck) which was incubated at 25°C for 5 days.

The amount of free fatty acids was determined by titration according to the method of Castberg, Solberg and Egelrud (1975) modified by Sørensen and Samuelsen (1996). Lipolysis was expressed as μmol free fatty acid (FFA) per 100 g butter fat. Each experiment was carried out in triplicate.

2.5. Determination of proteolytic activity

Using casein (g L^{-1}: Hammerstein casein (Merck), 10.0, $Ca(OH)_2$ (Merck), 0.30, $CaCl_2$, 0.20, agar–agar (Merck), 15.0; pH 5.5) as substrate the proteolytic activity was determined at 10°C by an agar diffusion test as described previously (van den Tempel & Jakobsen, 1998). Breakdown of casein monitored by high-pressure capillary electrophoresis (HPCE) was performed as described by Hansen and Jakobsen (1998). Yeast inocula were grown in MYGP broth at 25°C for 48 h. The concentration of the cells was estimated by the use of a counting chamber at 400× magnification (Awioskop microscope, Zeiss, Oberkocken, Germany). Casein medium was prepared from 37.5 g Czapek-broth (Oxoid) dissolved in 1000 mL 0.1 M phosphate buffer, pH 5.5. After autoclaving, 1 g of irradiated (10 KGY) casein nach Hammerstein (Merck) was added aseptically. The casein medium was inoculated with yeast at a rate of 10^5 cfu mL^{-1} and incubated at 10°C in a shaking water bath at 200 rpm. Samples were taken after 10 days of incubation at 10°C after reaching a final concentration of approximately

5×10^7 cfu mL^{-1}. The samples were prepared and analysed according to method by Larsen et al. (1998). Before performing the HPCE analysis the pH was measured showing a stable pH at 5.5, during the experiments. Examinations were made in duplicate.

2.6. Determination of enzyme profiles

The enzyme profiles of the strains of *Debaryomyces hansenii*, *Yarrowia lipolytica* and *Penicillium roqueforti* were determined with the API ZYM system (bioMerieux, Marcy l'Etoile, France), allowing the detection of extracellular alkaline and acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β glucosaminidase, lipase, 2 esterases, 2 proteases, 3 peptidases and 7 glycosidases. The yeasts and the strains of *P. roqueforti* were grown on Potato Dextrose Agar (Merck) and Czapek Agar (Difco), respectively, and suspensions (10^5 cfu mL^{-1}) in sterile distilled water were added to the microtubes of the API galleries, incubated at 37°C and read after 4 and 24 h. Level of enzymatic activity was expressed on a 0–5 scale, corresponding to the range of 0–40 nanomoles of substrate hydrolysed. The API ZYM test was carried out according to the instructions of the supplier.

2.7. Interactions between Debaryomyces hansenii and Yarrowia lipolytica and commercial strains of Penicillium roqueforti

Interactions were performed on cheese agar (4% NaCl, pH 5.5) as described by Larsen et al. (1998). Yeast inocula, cell-free extracts and inocula of *P. roqueforti* conidia were prepared according to Hansen and Jakobsen (1998).

A spore suspension of *P. roqueforti*, 0.1 mL (10^5 cells mL^{-1}) was spread onto the surface of cheese agar and left for drying at room temperature for 30 min. Three spots of 10 µL yeast suspension (10^5 cfu mL^{-1}), culture supernatant or cell-free extract were made on each plate, incubated for 14 d in the dark at 10°C. Interactions were recorded by monitoring visible stimulation or inhibition of growth and sporulation of *P. roqueforti* in or around the spot. The examinations were carried out in duplicate.

2.8. Pigment formation caused by Debaryomyces hansenii and Yarrowia lipolytica

Cheese agar (pH 5.5) was prepared as described above. *Debaryomyces hansenii* and *Yarrowia lipolytica* were grown in MYGP broth for 48 h at 25°C and inoculated on the cheese agar (10^5 cfu mL^{-1}). Control plates were inoculated with sterile MYGP broth. The plates were incubated at 10°C for 4 weeks and examined visually for pigment formation. Pigment development on cheese agar media with 1% (w/v) L-tyrosine (Merck) was also tested according to the method of Carreira, Paloma and Loureiro (1998).

3. Results

3.1. Growth characteristics

All strains were able to grow at pH from 4.0 to 6.0 at 10°C (a_w 0.99), the ripening temperature of Danablu (results not shown). At the same temperature the strains of *Debaryomyces hansenii*, were able to grow at a_w 0.92–0.99 whereas the strains of *Yarrowia lipolytica* were highly affected by low a_w and no growth was observed at $a_w = 0.94$ and 0.92 (results not shown).

3.2. Assimilation of lactose, galactose, lactate and citric acid

Lactose and galactose were assimilated by all strains of *Debaryomyces hansenii*. Assimilation of lactose by *D. hansenii* was strongly inhibited by salt contents higher than 4–6% (w/v). Only one strain was able to assimilate lactose at 8 and 10% (w/v) NaCl. Assimilation of galactose was less affected by NaCl and occurred for almost all strains at 12% NaCl (w/v) and two strains of *D. hansenii* were able to assimilate galactose at 14% NaCl (w/v). The strains of *Yarrowia lipolytica* did not assimilate lactose and galactose. All strains of the three yeast species assimilated lactate and citrate (Table 1), except one strain of *D. hansenii*. It is seen that the assimilation of lactate by *Y. lipolytica* was strongly inhibited by NaCl concentrations higher than 4% (w/v) whereas the strains of *D. hansenii* were able to assimilate lactate at 14% (w/v) NaCl (Table 2). One of the six strains of *D. hansenii* (CBS 164) was not able to assimilate citrate whereas all strains of *Y. lipolytica* assimilated citrate but they were more sensitive to NaCl than strains of *D. hansenii*.

3.3. Lipolytic and proteolytic activity

All strains of *Debaryomyces hansenii* and *Yarrowia lipolytica* hydrolysed tributyrin with *Y. lipolytica* having the highest activity at 10°C (results not shown).

Table 1
Assimilation of lactose and galactose at different concentrations of NaCl (w/v) for six isolates of *Debaryomyces hansenii* at 10°C and pH 5.5 *Debaryomyces hansenii*

NaCl % w/v	0%	2%	4%	6%	8%	10%	12%	14%
Lactose	6/6[a]	6/6	6/6	4/6	1/6	1/6	0/6	0/6
Galactose	6/6	6/6	6/6	6/6	6/6	6/6	5/6	3/6

[a]Number of isolates found positive over the total number of strains examined.

As seen from Table 3 *D. hansenii* showed little if any release of free fatty acids (FFA) from butterfat at 10°C. The table also shows that the lipolytic activity of *Y. lipolytica* is strong with variations between isolates. The activity is reduced in the presence of 4% (w/v) NaCl but still substantial.

In the agar diffusion test *D. hansenii* was not able to hydrolyse casein at 10°C whereas four of the strains of *Y. lipolytica* were positive (results not shown). According to the HPCE the four positive strains, CBS 2075, FB14, 30 and 132 were able to degrade all the casein components as shown for *Y. lipolytica* (CBS 2075) in Fig. 1. The figure also demonstrates that *D. hansenii* (CBS 164) did not degrade any casein components at 10°C. The same result was obtained for the other strains of *D. hansenii*. For *D. hansenii* hydrolysis of casein was observed at 25°C after extended incubation (results not shown).

3.4. Enzyme profiles

The enzyme profiles of *Debaryomyces hansenii* and *Yarrowia lipolytica* determined by the API ZYM test showed a wide but very similar spectrum of enzymes (Fig. 2) and differences were limited to Naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-glucosidase. *Penicillium roqueforti* showed a profile related to the yeasts, but *P. roqueforti* strains were more variable and weaker in peptidase activity, than the yeasts. *P. roqueforti* Roq 1, a strain with low proteolytic activity (Larsen et al., 1998) showed no peptidase activity.

Table 2
Assimilation of lactate and citrate at different concentrations of NaCl (w/v) for isolates of *Debaryomyces hansenii* and *Yarrowia lipolytica* at 10°C and pH 5.5

NaCl % w/v	0%	2%	4%	6%	8%	10%	12%	14%
Debaryomyces hansenii								
Lactate	6/6[a]	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Citrate	5/6	5/6	5/6	4/6	4/6	4/6	4/6	3/6
Yarrowia lipolytica								
Lactate	6/6	6/6	5/6	3/6	1/6	0/6	0/6	0/6
Citrate	6/6	5/6	3/6	4/6	0/6	0/6	0/6	0/6

[a]Number of isolates found positive over the total number of strains examined.

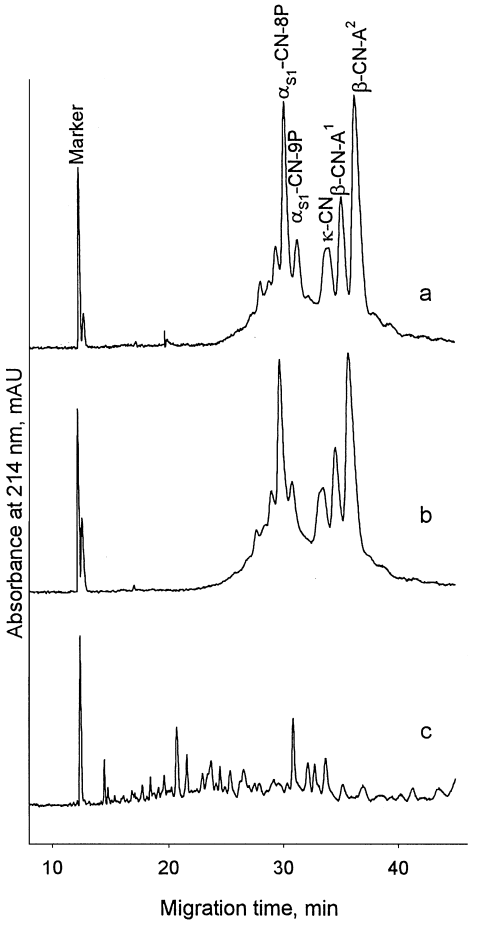

Fig. 1. CE profiles, absorbance at 214 nm versus time, of casein nach Hammerstein in Czapek–Dox broth at pH 5.5, control (a); inoculated with *Debaryomyces hansenii* — CBS 164 (b) and *Yarrowia lipolytica* (c) after incubation for 10 days at 10°C.

Table 3
The amount of free fatty acids released (μmol FFA/100 g fat) after 10 days of incubation at 10°C for six strains of *Debaryomyces hansenii* and six strains of *Yarrowia lipolytica* in butterfat emulsions, at pH 5.5 with 0 or 4% (w/v) NaCl added. The results are given as average and standard deviation of three replications

Debaryomyces hansenii NaCl % (w/v)	CBS 164	FB1	FB2	3	78	135
0	n.d[a]	12 ± 0	12 ± 0	n.d	n.d	12 ± 0
4	n.d	n.d	n.d	n.d	n.d	n.d
Yarrowia lipolytica						
NaCl % (w/v)	CBS 2075	FB14	30	77	132	138
0	496 ± 11	606 ± 10	551 ± 63	1004 ± 0	324 ± 0	673 ± 64
4	239 ± 11	165 ± 11	233 ± 10	386 ± 9	76 ± 10	120 ± 9

[a]not detectable.

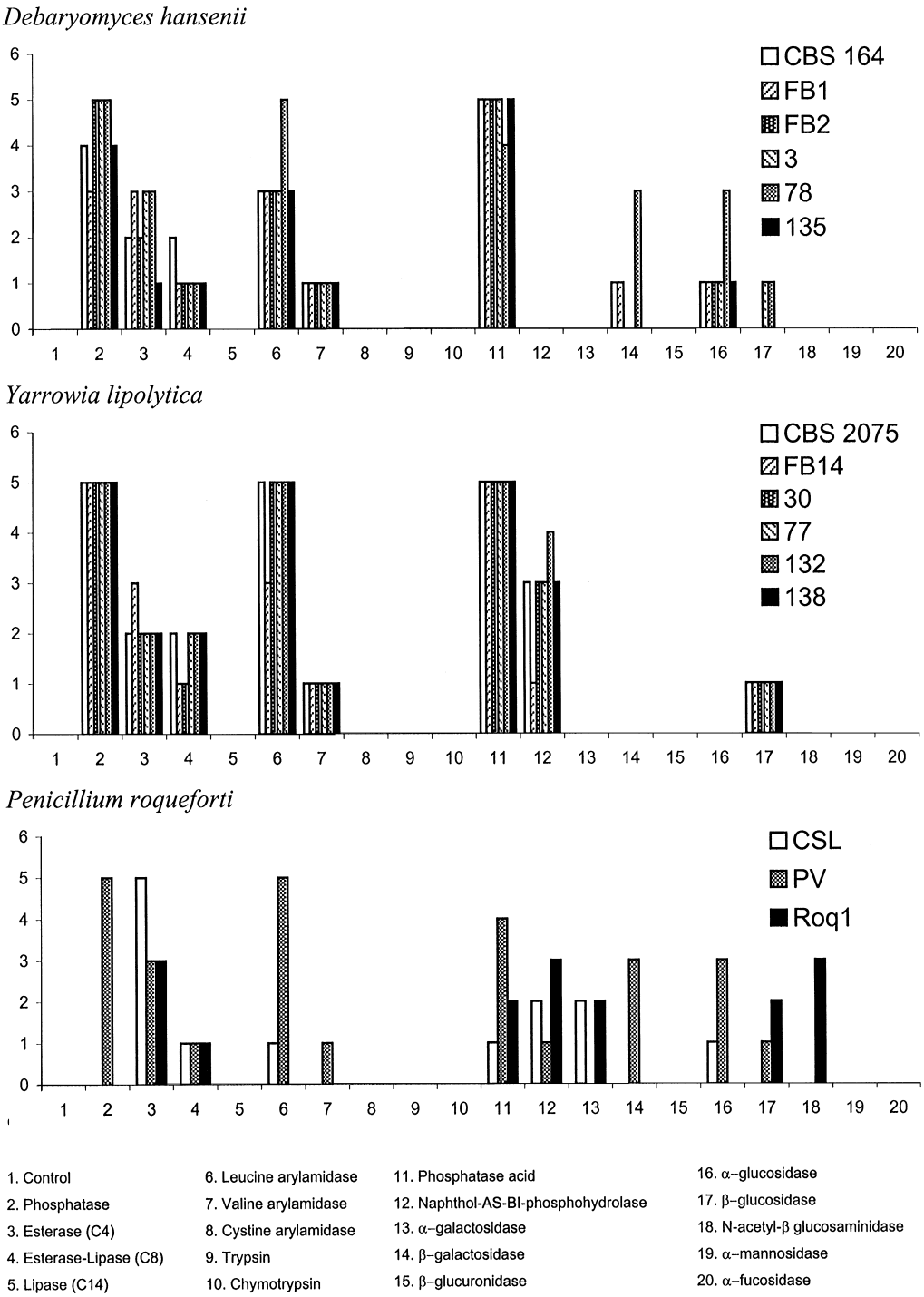

Fig. 2. Enzyme profiles (API ZYM, bioMerieux, marcy l'Ecoile, France) of 6 strains of *Debaryomyces hansenii* and *Yarrowia lipolytica*, respectively, and 3 strains of *Penicillium roqueforti*.

3.5. Interactions between strains of Debaryomyces hansenii and Yarrowia lipolytica and strains of Penicillium roqueforti

In several occasions, *D. hansenii* and *Y. lipolytica* were found to be inhibitory against strains of *P. roqueforti* on cheese agar as shown in Table 4. The inhibitory potential against *P. roqueforti* was particularly pronounced for strains of *Y. lipolytica* especially CBS 2075, FB 14 and 77. Among the strains of *P. roqueforti* PV was the most resistant and CLS and Roq 1 the most sensitive. Inhibition was not observed when yeast culture supernatants or the cell free extract were used (results not shown).

3.6. Pigment formation by Debaryomyces hansenii and Yarrowia lipolytica

The pigment production in cheese agar with and without added tyrosine is shown in Table 5. The strains of *D. hansenii*, except CBS 164, did not show any pigment production after 28 days of incubation at 10°C. However, all strains of *Y. lipolytica* in most cases showed strong pigment production with variations between species. In cheese agar with added 1% tyrosine, pigment formation in general was more pronounced. The pigment produced was red-brownish or brownish. The pigment observed for the one isolate of *D. hansenii* was similar in colour to the pigment produced by *Y. lipolytica*.

Table 4
Inhibition of *Penicillium roqueforti* by strains of *Debaryomyces hansenii* and *Yarrowia lipolytica* after 14 days of incubation at 10°C on cheese agar[a]

	Isolates of *Penicillium roqueforti* surface spread (10^5 cells/ml) on to cheese agar		
Spot (10^5 cells^{-1}ml)	PV	CSL	ROQ 1
Debaryomyces hansenii CBS 164	+	+	+
FB1	0	+ +	+
FB2	0	+	+
3	+	0	+
78	0	+	+
135	0	+ +	+ +
Yarrowia lipolytica CBS 2075	+ +	+ + +	+ + +
FB14	+ +	+ + +	+ + +
30	+ + +	+ +	+ +
77	+ + +	+ + +	+ +
132	+	+ + +	+ +
138	+ +	+ + +	+ +

[a]The results for interactions between moulds and yeast are expressed as:
[0] no inhibition of mycelial growth and sporulation, [+] weak inhibition, sparse mycelial growth and sporulation, [++] medium inhibition, sparse mycelial growth and no sporulation, [+++] strong inhibition, no growth.

4. Discussion

The microenvironment in Danablu during processing and ripening is characterised by a minimum value of pH 4.6 gradually increasing to values just above 6.0 during the 4–6 weeks of ripening at 10–12°C. Corresponding a_w values in the centre and periphery, are about 0.99 and 0.87 (NaCl 1–7% w/w) after 1 week, reaching a level of 0.94 and 0.92 (NaCl 2–4.5% w/w), respectively, after 5 weeks (unpublished results).

The growth characteristics of the strains of *Debaryomyces hansenii* investigated show that the environmental conditions prevailing in Danablu during ripening at 10°C will support growth of this yeast. Growth of *Yarrowia lipolytica* is restricted by the levels of NaCl ($a_w = 0.92$ and 0.94), indicating that the growth can take place primarily in the centre of Danablu during the early stages of ripening until the salt gradient is equalized throughout the cheese after approximately 4 weeks of ripening.

All strains of *D. hansenii* and *Y. lipolytica* assimilated lactate and all except one strain of *D. hansenii* assimilated citrate. By assimilation of these acids the yeasts will

Table 5
Pigment production after 28 days of incubation (10 and 25°C) on cheese agar with and without 1% (w/v) L-tyrosine

	Cheese agar without tyrosine		Cheese agar with 0.1% (w/v) -tyrosine	
Strain	10°C	25°C	10°C	25°C
Debaryomyces hansenii (CBS 164)	−[a]	−[a]	+[d]	+ +[d]
FB1	−[a]	−[a]	−[a]	−[a]
FB2	−[a]	−[a]	−[a]	−[a]
3	−[a]	−[a]	−[a]	−[a]
78	−[a]	−[a]	−[a]	−[b]
135	−[a]	−[a]	−[a]	−[a]
Yarrowia lipolytica (CBS 2075)	+ +[f]	+ + +[e]	+ + +[d]	+ + +[e]
FB14	+[f]	+[e]	+ +[d]	+ +[e]
30	+[f]	+ +[e]	−[c]	+ + +[e]
77	+[f]	+[e]	+ +[d]	+[e]
132	−[a]	−[b]	+[d]	+ +[e]
138	+ +[e]	+ +[e]	+ + +[d]	+ + +[e]

Colour intensity of media: weak +, medium + + and strong + + +.
[a]No pigment formation in medium and white colony.
[b]No pigment formation in medium but brownish colony.
[c]No pigment formation in medium but yellowish colony.
[d]Reddish medium and white colony.
[e]Brownish medium and brownish colony.
[f]Red-brownish medium and white colony.

modify the microenvironment of the Danablu by increasing the pH. Further *D. hansenii* can utilise lactose and galactose at NaCl levels of 8 and 14%, respectively, which will reduce the risk of undesired fermentations especially in regard to non-starter lactic acid bacteria.

As reported earlier (van den Tempel & Jakobsen, 1998) *D. hansenii* and *Y. lipolytica* hydrolyse tributyrin at 30°C, with isolates of *Y. lipolytica* showing the highest activity. However, the present study has demonstrated that hydrolysis of butterfat by *D. hansenii* is very limited at 10°C and pH 5.5, even though yeast growth still takes place. Besides a significant effect of temperature, the pH also seems to have an influence on the lipolytic activity of *D. hansenii* as reported by Sørensen (1997) and Sørensen and Samuelsen (1996) for a strain of *D. hansenii* used for meat fermentation. The strains of *Y. lipolytica* were strongly lipolytic. The lipolytic activity of *Y. lipolytica* strains was effected by the low temperature at 10°C and addition of 4% NaCl, but a pronounced lipolysis could still be detected, suggesting a potential role in ripening of Danablu. By comparison with investigations done by Larsen and Jensen (1999) on different strains of *P. roqueforti*, it is indicated that the lipolytic activity of *Y. lipolytica* can be as strong as the activity of *P. roqueforti* strains under the conditions prevailing in Danablu. Strong lipolytic activity of yeasts has recently been demonstrated by Gobbetti, Burzigotti, Smacchi, Corsetti and Angelis (1997) who found that lipolysis in Gorgonzola cheese depended less on the activity from *P. roqueforti* and more on the surface microflora, where yeasts were found at high numbers.

In this investigation *D. hansenii* was found to be non-proteolytic at 10°C after 10 days of incubation. This is contrary to results showing *D. hansenii* from other habitats to be weakly proteolytic at 10°C (Lagace & Bisson, 1990; Kobatake, Kreger van-Rij, Plácido & van Uden, 1992). In previous studies *D. hansenii* strains FB1 and FB2 showed proteolytic activity after 3 weeks of incubation at 25°C, with degradation of all components of the casein (unpublished results). These results seem to indicate that *D. hansenii* is proteolytic but not contributing in any significant way to the proteolytic activity during ripening of Danablu at 10–12°C. The strong proteolytic activity by strains of *Y. lipolytica* is well documented (Ahearn, Meyers & Nichols, 1968; Foda & El-Din, 1979; Kobatake & Kurata, 1983; Wyder, 1998). According to Kobatake et al. (1992) *Y. lipolytica* is proteolytic in a broad temperature range, which agrees with the results obtained in the present study, showing that *Y. lipolytica* can degrade all components of the casein at 10°C and 4% NaCl. The strong proteolytic activity of *Y. lipolytica* at the environmental conditions similar to Danablu suggest a possible contribution to the ripening of Danablu. Use of *Y. lipolytica* as a starter could give rise to an intensification of the flavour (Adda, Gripon & Vassal, 1982) and acceleration of the textural development (Guerzoni et al., 1998). The aminopeptidase activity demonstrated for *Y. lipolytica* as well as *D. hansenii* could also play a role during cheese ripening, but it remains to be investigated for environmental conditions relevant to Danablu.

In the present investigations strains of *P. roqueforti* and the strains of *D. hansenii* and *Y. lipolytica* showed strain-specific interactions. The strains of *Y. lipolytica* were all inhibitory towards mycelial growth and sporulation of *P. roqueforti*. As inhibition of *P. roqueforti*, was not seen when using culture supernatants and cell-free extract of *Y. lipolytica* the mechanism of inhibition seems to be explained by competitions on nutrients on cheese agar. The inhibition of *P. roqueforti* by *D. hansenii* was less pronounced and in some cases *D. hansenii* did not affect growth and sporulation of *P. roqueforti*. Other yeast–mould interactions have been described (Björnberg & Schnürer, 1993; Droby, Chalutz, Wilson & Wiwniewski, 1989) which together with the present results support the statement of Janisiewicz (1988) that a rapid growth of yeasts may restrict the availability of nutrients essential for the moulds. However, further studies are needed to fully understand the mechanisms of interactions between potential yeast starter cultures and *P. roqueforti* in Danablu.

Pigment formation in mould-ripened cheeses is reported to be associated with yeasts, including *Y. lipolytica* and *D. hansenii* (Nichol & Harden, 1993; Nichol, Harden & Tuckett, 1996). Pigmentation in Danablu occurs occasionally and is not limited to the surface of the cheese as pigment formation also has been observed in the centre of the cheese (unpublished results). The pigmentation primarily seems to be caused by the oxidation of tyrosine to melanin in reactions catalysed by tyrosinase (Weichhold, Seiler, Busse & Klostermeyer, 1988; Carreira et al., 1998). In the present work only one strain of *D. hansenii* produced pigment on cheese agar with 1% (w/v) tyrosine. This corresponds with results of Nichol et al. (1996) who found low tyrosinase activity in *D. hansenii*. All strains of *Y. lipolytica* appeared to have a high tyrosinase activity with differences at subspecies level, which also agrees with Nichol et al. (1993) and Carreira et al. (1998). These results emphasise that, careful selection of starter culture strains by screening strategies to differentiate pigment producing yeasts, is necessary to avoid cheese defects.

In conclusion the potential use of *D. hansenii* as a starter culture seems to be linked with its osmotolerance and good growth in Danablu together with its assimilation of lactate and citric acid, lactose and galactose modifying the microenvironment in the cheese to the benefit of *P. roqueforti* and protection of the cheese against undesired carbohydrate fermentations. For *Y. lipolytica* the potential role of starter culture is linked with early lipolysis at a time when the lipase from *P. roqueforti* is not present in any significant amounts, but it may also contribute to the proteolysis during maturation.

Acknowledgements

The assistance of Kristian Rothvig Kristiansen and Anni Nielsen who carried out the CE analysis is gratefully acknowledged. The authors are grateful to Per Christensen for excellent technical assistance. We wish to thank MD Foods, Aarhus, Denmark for donating Danablu for the experimental work. This work is a part of a Danablu project supported by the Danish Ministry of Food Agriculture and Fisheries and the Danish Dairy Board, and carried out within the framework of the Centre of Advanced Food Studies, the Royal Veterinary and Agricultural University, Copenhagen.

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