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The growth, properties and interactions of yeasts and bacteria associated with the maturation of Camembert and blue-veined cheeses

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Abstract

The growth of yeasts and bacteria were monitored during the maturation of Camembert and blue-veined cheese produced in Australia. Yeasts were prominent throughout maturation, growing to 10^5–10^9/g, depending on the manufacturer. *Debaryomyces hansenii* predominated, but there were lesser, inconsistent contributions from *Yarrowia lipolytica*. Of the non-lactic acid bacteria, *Acinetobacter* species were significant during the maturation of Camembert but not blue-veined cheeses, and grew to 10^6–10^8 cfu/g. *Staphylococcus* and *Micrococcus* species were consistently isolated from the cheeses with *Staphylococcus xylosus* growing to 10^5–10^9 cfu/g, depending on the product. Lactic acid bacteria (10^7–10^9 cfu/g) were present throughout maturation but were not identified. Interactions between the various yeasts and bacterial isolates were examined. Several strains of *D. hansenii* exhibited killer activity but not against *Y. lipolytica*. None of the yeasts were antagonistic towards the bacteria but some strains of *D. hansenii* enhanced the growth of *Y. lipolytica* and *S. xylosus*. The yeast and bacterial isolates exhibited various degrees of extracellular proteolytic and lipolytic activities.

Keywords: Cheese maturation; Yeasts; Bacteria; *Debaryomyces hansenii*; *Yarrowia lipolytica*; *Acinetobacter*; *Staphylococcus*; *Micrococcus*

1. Introduction

The production of mould-ripened cheeses, such as the Camembert and blue-veined varieties, involves a maturation stage that is characterised by the growth of a complex ecology of yeasts, bacteria and filamentous fungi. The microbiological interactions and associated biochemical activities that occur during this stage determine product acceptability and value through their impacts on sensory quality, shelf life and safety (Olson, 1995; Banks, 1998; Stanley, 1998a,b; Fleet, 1999). The maturation flora consists of "carry-over" lactic acid bacteria from milk fermentation, inoculated *Penicillium* and *Geotrichum* species and secondary or non-starter microorganisms. Essentially, this secondary flora originates as natural contaminants of the process and comprises a diversity of yeast and bacterial species that may grow to populations as high as 10^9 cfu/g. Numerous studies have now identified the various species of yeasts and bacteria that contribute to the secondary flora and these are discussed in reviews by Fleet (1990, 1999), Jakobsen and Narvhus (1996) and Stanley (1998a,b). However, there is a need for

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further research that better defines the growth profiles of individual species throughout the time frame of maturation, the key properties of these species (e.g. proteolytic and lipolytic activities) that are likely to affect product quality, and the potential for ecological interactions between the different species. Such information is needed to optimise process conditions so that the growth of undesirable species might be prevented, or the growth of desirable species could be promoted. Also, it would help to identify species that might be developed as specialised starter cultures for cheese maturation (Martin et al., 1999; Wyder and Puhan, 1999).

This study reports the growth profiles of species of yeasts and bacteria throughout the production of Camembert and blue-veined cheeses at several commercial operations in Australia. The extracellular proteolytic and lipolytic activities of the principal species are reported, along with the potential for individual species to influence the growth and survival of other species.

2. Materials and methods

2.1. Microbiological analysis of cheeses

Four commercially produced cheeses were examined. One Camembert (A) was produced at a factory in the state of New South Wales, while the other Camembert (B) was produced in the state of Queensland. Blue-veined cheese (C) was produced in Queensland and blue-veined cheese (D) was produced in the state of Victoria. The cheeses were analysed for populations of lactic acid bacteria, total aerobic bacteria and yeasts at regular intervals throughout the entire process of production—from milk fermentation through maturation until packaging. Duplicate production batches of each cheese were examined, with analyses of each batch being done in duplicate. For each batch, inner and outer sections of the cheese curd were examined. There were minor variations in populations between each batch of the one cheese, but the overall ecological trends were similar. The data presented are the average of analyses for a single batch.

Cheese samples (10 g) were suspended in 0.1% peptone solution and blended in a Colworth Stomacher 400 for 1–2 min. The suspension was diluted in 0.1% peptone solution and examined for microbial populations by the spread plate procedure. Lactic acid bacteria were enumerated by plating onto MRS (Oxoid, Melbourne, Australia) and M17 (Oxoid) agar media, that were supplemented with 50 mg/l of cycloheximide to suppress the growth of yeasts and moulds. Plates were incubated microaerobically for 7 days at 30 °C. Total aerobic bacteria were enumerated by plating onto (i) Tryptone Soya Agar (TSA, Oxoid) that had been supplemented with 0.1% glucose, 4% w/v NaCl and 50 mg/l of cycloheximide and (ii) Mannitol Salt Agar (Oxoid) with cycloheximide. Plates were incubated for 4 days at 30 °C. Yeasts were enumerated by plating onto Malt Extract Agar (MEA, Oxoid) supplemented with 100 mg/l of oxytetracycline to suppress bacterial growth, and 50 mg/l of biphenyl to restrict the growth of filamentous fungi. Plates were incubated for 7 days at 20–25 °C.

Since Camembert and blue-veined cheeses contain significant concentrations of salt (NaCl), it was considered that they might harbour a salt-adapted microflora that would require salt-supplemented media for their isolation. We examined this possibility in a series of preliminary experiments using diluent and media supplemented with 4% NaCl, and found no statistically significant differences between populations obtained on supplemented and non-supplemented media. While not statistically significant, there was some evidence of slightly increased recovery of aerobic bacteria on TSA supplemented with NaCl. Incorporation of biphenyl into MEA did not affect yeast counts, but greatly assisted yeast isolation by restricting mould growth (Addis et al., 1998).

Several representative colonies from each plate were isolated, purified and maintained for identification.

2.2. Identification of microorganisms

The sampling and analytical protocol gave many hundreds of bacterial and yeast isolates for each cheese. For example, studies of cheese A generated 440 yeast and 751 bacterial isolates, while cheese B gave 571 yeasts and 1057 bacterial isolates. Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) of whole cell proteins was used to screen the isolates for similarities in their profiles.

After optimisation of cell extraction and electrophoretic running conditions, according to Pot et al. (1994), it was found that most isolates gave consistent, distinguishable profiles that enabled their assignment to a small number of groups, representatives of which had the same genus and species identification. With this approach, it was possible to substantially decrease the number of isolates requiring formal, detailed identification. Thus, for cheese A, for example, the 751 bacterial isolates were reduced to 79 requiring identification and the 440 yeast isolates were reduced to 29.

Lactic acid bacteria, accepted as the Gram-positive, catalase-negative colonies on plates of MRS and M17 agar, were not subject to any further identification. Aerobic bacterial isolates from plates of TSA and MSA were analysed for morphology, Gram and catalase reactions, and then identified to species using ATB32 Staph kits (BioMerieux, France) for *Staphylococcus* and *Micrococcus* species and Biolog GN test plates (Biolog, California) and ATB 32GN and API 20 NE test strips (BioMerieux) for *Acinetobacter* species. After basic morphological analysis (Kurtzman and Fell, 1998), yeasts were identified to species using the Biolog YN and ATB 32C (BioMerieux) systems as described previously (Praphailong et al., 1997).

2.3. Extracellular proteolytic and lipolytic activities

Proteolytic activity was determined by spot culture of microorganisms on Skim Milk Agar (Lee and Kraft, 1992) at 30 °C for bacteria and 25 °C for yeasts, and noting the clear zones of skim milk hydrolysis (5-mm margins, strong activity; 1–2.5-mm margins, weak activity). Lipolytic activity was similarly examined by culture on Tributyrin Agar (Oxoid) and Butterfat Agar (Smith and Haas, 1992). These media were adjusted to give pH values between 6.0 and 7.5 and to have NaCl concentrations from 0.5% (w/v) (control) to 7.2% (w/v). These variables were examined to simulate conditions in the cheese from where the organisms were isolated.

2.4. Microbial interactions

The spot-on-lawn assay (Piddock, 1990; Hoover and Harlander, 1993) was used to examine interactions between yeast and bacterial cultures. The basal media used were either MEA, TSA or Brain Heart Infusion Agar (Oxoid). The medium was prepared and maintained in a molten (50 °C) state. The species under test was seeded at 10^5–10^6 cfu/ml into the molten medium, which was then poured into a petri plate. After cooling and solidification, the medium was spot-inoculated on the surface with another species. The seed and spot inoculum species were grown up as fresh (24–48 h) cultures in either Malt Extract Broth (Oxoid) for yeasts or Tryptone Soy Broth (Oxoid) or Brain Heart Infusion Broth (Oxoid) for bacteria. Inoculated plates were incubated at either 25 °C for 4 days (yeasts) or 30 °C for 2 days (bacteria). Inhibition of the seeded organism by the spot-inoculated organisms was indicated by a clear zone surrounding growth of the spot culture. Stimulated or enhanced growth of the seeded organism was evidenced by increased biomass density surrounding the spot culture. In some cases, yeast and bacterial strains other than those isolated from the cheeses already described were used in these interactive studies. These cultures were obtained from the collection of the Department of Food Science and Technology, The University of New South Wales. Except for the pathogens *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*, these organisms had been isolated from cheeses.

Determination of the killer interaction between yeast isolates followed the spot-on-lawn procedure as previously described, but MEA, pH 5.25, was used as the basal medium (Heard and Fleet, 1987). Killer interaction assays were also conducted in MEA supplemented with NaCl at 2.5%, 5.0% and 7.0% (w/v).

3. Results

3.1. Growth of microorganisms during production of Camembert cheese

The pasteurised milk for product A contained about 10^3 cfu/ml of aerobic bacteria but lactic acid bacteria were not detectable ($< 10^2$ cfu/ml). The milk was inoculated with 10^6 cfu/ml of starter lactic acid bacteria (*Lactococcus lactis*), which grew to

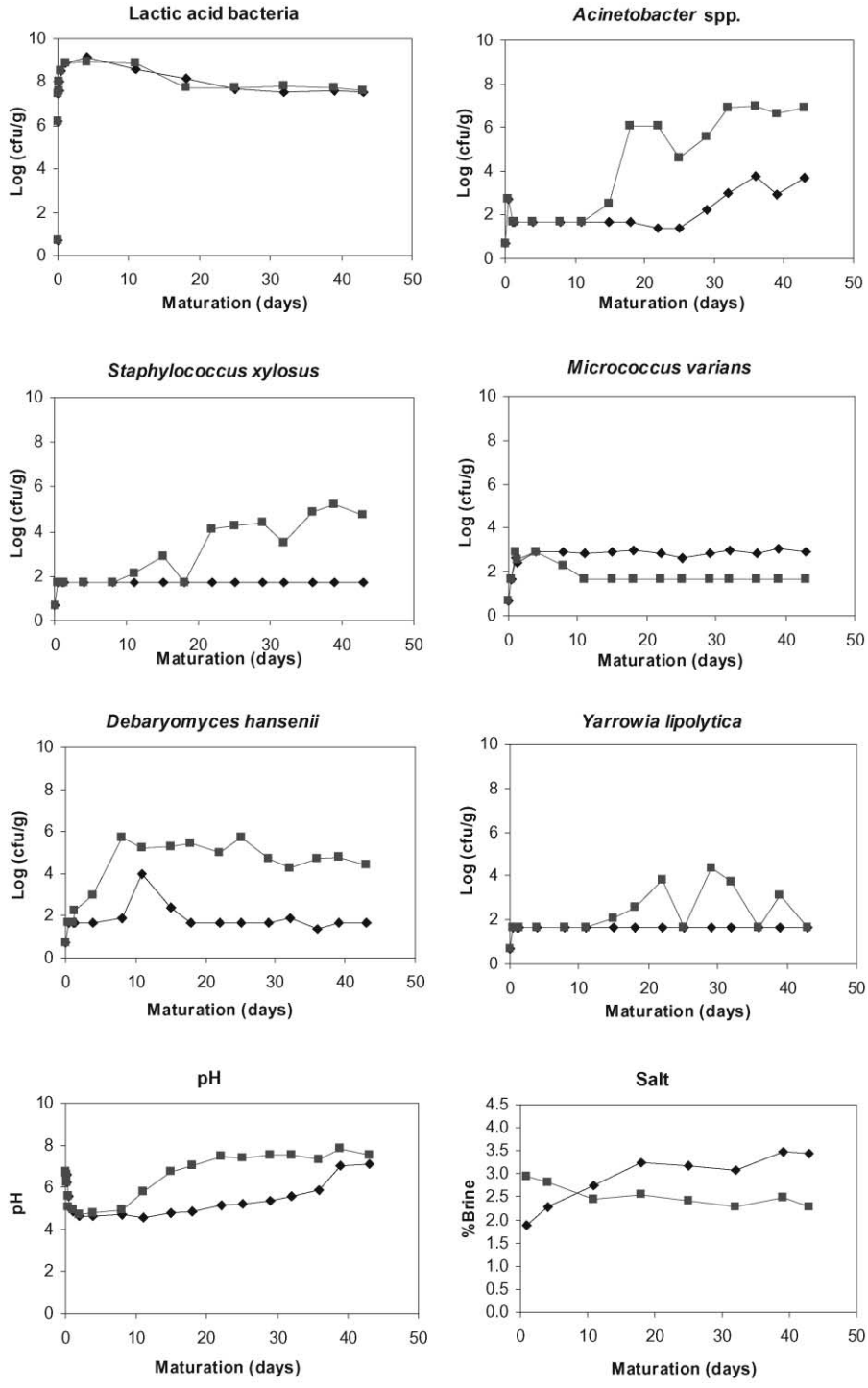

Fig. 1. Changes in the populations of bacteria and yeasts, pH and NaCl content during the maturation of Camembert cheese A. Inner curd (♦); outer curd (■).

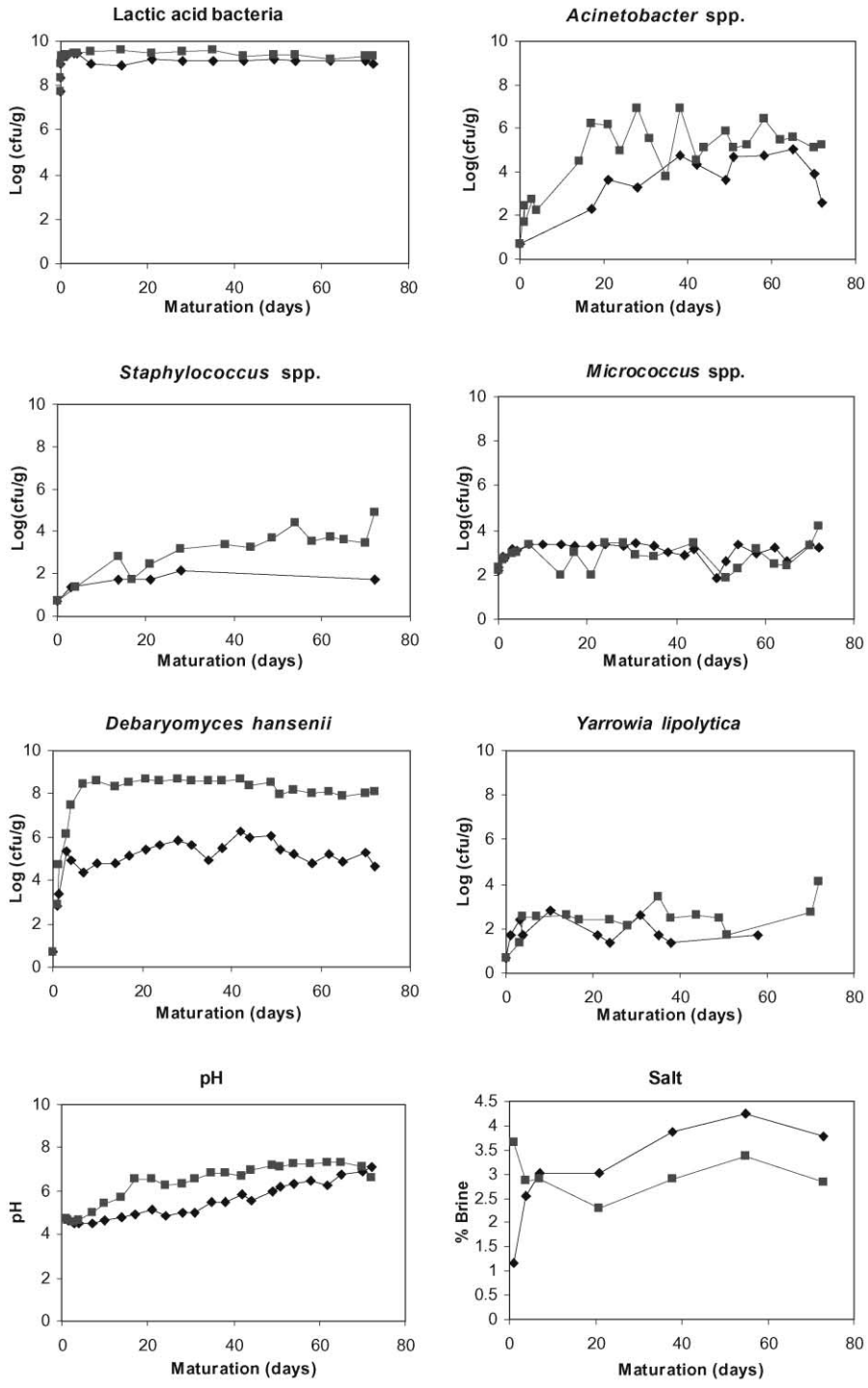

Fig. 2. Changes in the populations of bacteria and yeasts, pH and NaCl content during the maturation of Camembert cheese B. Inner curd (♦); outer curd (■).

about 10^8 cfu/ml during milk fermentation and curd production (first 10 h). Further growth of lactic acid bacteria to approximately 10^9 cfu/g occurred during the first 4 days of maturation, after which, there was a gradual decrease to 10^7 cfu/g (Fig. 1). Total aerobic bacteria increased during maturation from initial populations of 10^3 to 10^6–10^7 cfu/g for outer curd samples and 10^3–10^4 cfu/g for inner curd samples. Some inconsistently isolated species were *Klebsiella oxytoca*, *Citrobacter freundii*, *Pseudomonas putida* and *Bacillus* spp. However, their populations were relatively low and did not exceed 10^2–10^3 cfu/g. *Acinetobacter genospecies* 15 (Biolog identification) or *A. lwoffii* (API 20 NE identification) were consistently isolated throughout maturation and increased to populations of 10^6–10^7 cfu/g during the mid–later stages (Fig. 1). Species of *S. xylosus* and *Micrococcus varians* were also consistently isolated, with the *Staphylococcus* species showing some tendency to grow (Fig. 1).

Yeasts were not detected in the product until 2–4 days into maturation. Thereafter, they grew to 10^5–10^6 cfu/g at the outer curd. *Debaryomyces hansenii* was the dominant species but *Yarrowia lipolytica* was inconsistently isolated at much lower populations (Fig. 1).

The moulds, *Penicillium camembertii* and *Geotrichum candidum* were introduced into the cheese as starters at the stage of milk fermentation. They developed to about 10^7 cfu/g on the outer curd.

At the end of milk fermentation, curd pH was about 4.5. This value increased to pH 7.5 (outer curd) and pH 7.2 inner curd (Fig. 1). The salt (NaCl) content after brining was 3.0% (w/w) (outer curd) and 1.9% (w/w) (inner curd) and had equilibrated to 3.5% and 2.4%, respectively, by the end of maturation (Fig. 1).

The kinetics of microbial growth and changes to pH and NaCl content for Camembert B (Fig. 2) were similar to those observed for Camembert A. However, the populations of *D. hansenii* (10^8–10^9 cfu/g outer curd) were about 100-fold greater in Camembert B. *A. calcoaceticus* (10^6 cfu/g outer curd) was the most significant bacterial species, apart from the lactic acid bacteria. *M. luteus* and *M. roseus* were predominant among the micrococci. *S. xylosus* exhibited some growth in the outer curd and, occasionally, *S. saprophyticus* was isolated. *Bacillus* spp. were consistently isolated throughout maturation, but at low populations (10^2–10^3 cfu/g).

For both cheeses A and B, significant growth of *Acinetobacter*, *Staphylococcus* and *Micrococcus* species did not occur until after growth of the yeast, *D. hansenii*.

The lactic acid bacteria that were present during the maturation experiments of Camembert A and B were not identified. However, samples of these cheeses that were examined on other occasions showed a predominance (10^6–10^8 cfu/g) of *L. lactis* (starter culture) and *Enterococcus faecium* (cheese A) and *E. faecalis* (cheese B). On some occasions, *E. avium* and *E. faecalis* (10^5–10^6 cfu/g) were also obtained from cheese A.

3.2. Growth of microorganisms during production of blue-veined cheeses

Fig. 3 shows the evolution of bacteria and yeasts throughout the production of blue-veined cheese C. No *Acinetobacter* spp. were detected but growth of *S. xylosus* (10^8–10^9 cfu/g) and *Micrococcus* spp. (principally *M. varians*) was significant. Such growth occurred in the later stages of maturation, after growth of the yeasts, principally *D. hansenii* (10^8–10^9 cfu/g) and to a lesser extent *Y. lipolytica*. *P. roqueforti* was introduced into this cheese with the starter culture of lactic acid bacteria (*L. lactis*). It developed to 10^8 cfu/g throughout maturation and was the only mould species detected. The pH of the curd increased from 4.9 to 6.3 throughout maturation. The NaCl content after brining was 10.7% (w/w) (outer curd) and 3.4% (w/w) (inner curd), which equilibrated to 7.1% (w/w) and 6.8% (w/w), respectively, during maturation.

Only yeast species were identified during the maturation of blue-veined cheese D (data not shown). *D. hansenii* (10^8–10^9 cfu/g) predominated after 20 days of maturation, but *Y. lipolytica* was present at 10^6 cfu/g and *Candida zeylanoides* occurred at 10^4–10^5 cfu/g. The population of aerobic bacteria in cheese D increased to 10^9 cfu/g (outer curd) and 10^8 cfu/g (inner curd), but only after 40 days when the yeasts had grown. After an initial decrease from 10^9–10^{10} cfu/g, lactic acid bacteria remained at approximately 10^8 cfu/g throughout maturation (data

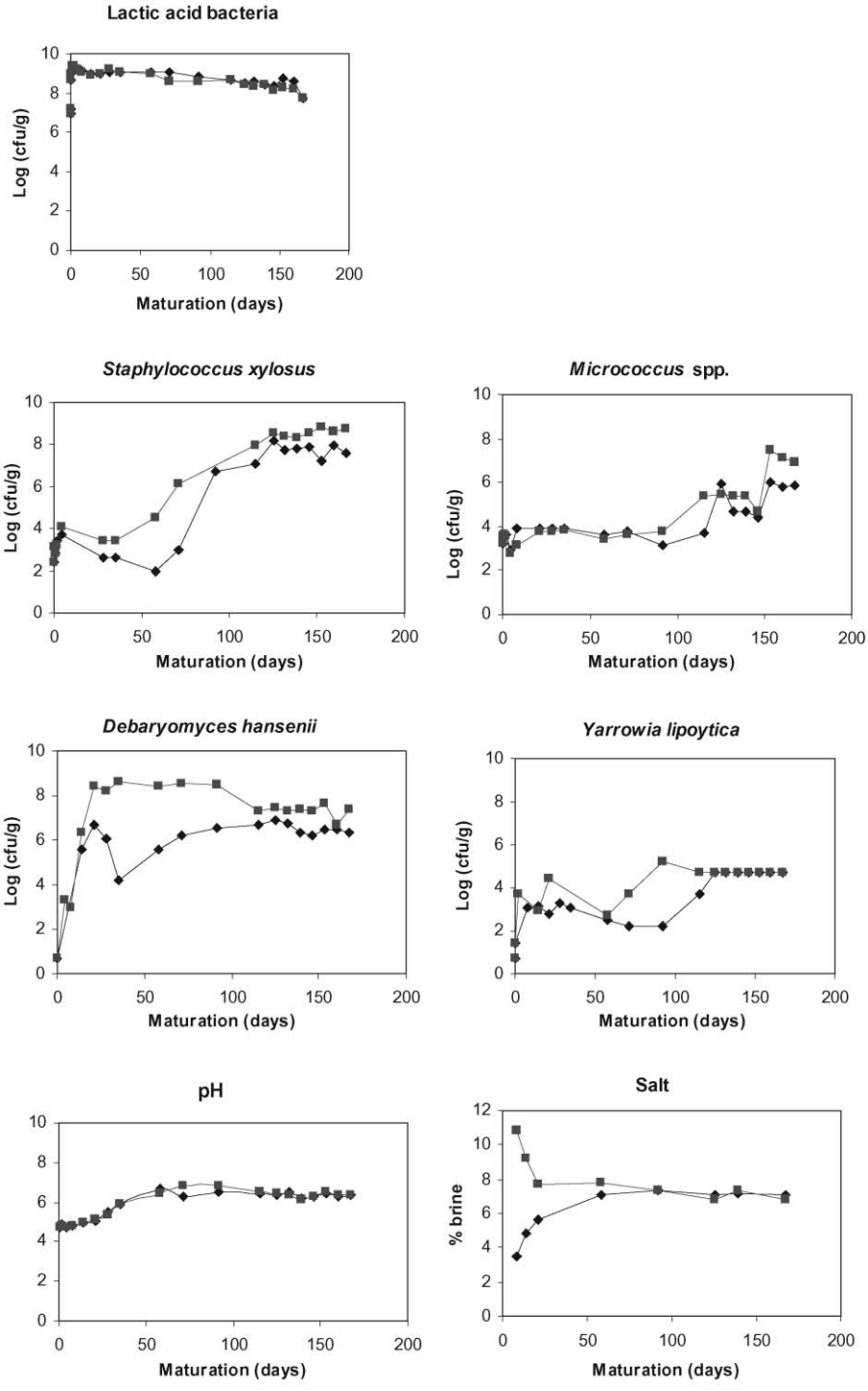

Fig. 3. Changes in the populations of bacteria and yeasts during the maturation of blue-veined cheese C. Inner curd (♦); outer curd (■).

not shown). In other studies, we have isolated *Lactobacillus* spp. from cheeses produced at this factory. For the non-lactic acid bacteria, populations of *Staphylococcus*, *Micrococcus* and *Bacillus* species were prevalent.

3.3. Proteolytic and lipolytic activities

Production of extracellular proteolytic and lipolytic activities by the microbial strains isolated during maturation is summarized in Table 1. None of the isolates of *D. hansenii* were clearly proteolytic, but they all exhibited lipolytic activity, which ranged from weak to strong on butterfat agar, depending on the strain. All isolates of *Y. lipolytica* were proteolytic, giving either strong to very strong reactions. Most *Y. lipolytica* were lipolytic, and gave weak to strong reactions. For some yeast isolates, inclusion of NaCl (2.5–7% w/v) in the assay medium decreased the strength of proteolysis or lipolysis. The *Acinetobacter* spp., which were predominant during the maturation of Camembert cheeses, were non-proteolytic, but approximately half of the isolates gave weak to strong lipolytic reactions on butterfat agar. Stronger lipolytic activities were observed on tributyrin agar. For some *Acinetobacter* strains, the presence of NaCl enhanced the lipolytic reaction. Most of the *Staphylococcus* and *Micrococcus* isolates were strongly proteolytic. The presence of NaCl enhanced the proteolytic reaction by some strains of *Staphylococcus* but decreased this reaction from some *Micrococcus* isolates. Some of the *Staphylococcus* and *Micrococcus* strains gave weak to strong lipolytic activities. Although isolates of *Bacillus* spp. were not numerically significant during maturation, some were notable for strong protease production.

Table 1
Extracellular production of protease and lipase activity by microorganisms isolated from cheeses during maturation

Species	Protease production	Lipase production
D. hansenii	0/55[a]	55/55[b]
Y. lipolytica	25/25	23/25
Acinetobacter spp.	0/29	15/29[b]
Staphylococcus spp.	46/64	12/64
Micrococcus spp.	34/37	2/37
Bacillus spp.	19/20	5/20

Data represent the number of positive strains out of the total number tested.
[a]Weak reactions.
[b]Weak to strong reaction.

3.4. Interactions between microorganisms

3.4.1. Yeast–yeast interactions

D. hansenii (10 strains), *Y. lipolytica* (five strains), *Kluyveromyces marxianus* (four strains) and *Saccharomyces cerevisiae* (two strains) were tested for killer interactions. Each strain was examined as either the killer or sensitive counterpart for every other strain. Of the 441 interactions examined, only 11 cases of the killer phenomenon were observed, and these were confined to a few strains of *D. hansenii* that inhibited a strain of *D. hansenii*, which had been isolated elsewhere (salted fish). Representatives of these killer strains were obtained from each of the cheeses A, B and C. These killer interactions were not observed when the culture medium was at pH 5.5 or higher, but were observed at NaCl concentrations from 0% to 7% w/v. However, inclusion of NaCl in the assay medium did not increase the number of killer interactions that were observed. Surprisingly, the agar plates used to determine killer activity revealed examples where the spotted yeast strain clearly enhanced growth of the strain that had been seeded into the basal medium. This property was evidenced by the increased density of growth of the seeded strain surrounding the colony of the spotted strain. Examples of this interaction were: enhancement of the growth of *Y. lipolytica* and *K. marxianus* by numerous (but not all) strains of *D. hansenii* (Fig. 4); and enhancement of the growth of *Y. lipolytica* and *K. marxianus* by *Sac. cerevisiae*. None of the strains of *Y. lipolytica* or *K. marxianus* enhanced the growth of other yeasts.

Another interesting observation was the distinctive change in the colony morphology of spot-inoculated strains of *Y. lipolytica* when cultured with *D. hansenii* as the seeded strain. Generally, the *Y. lipolytica* colonies were larger, with spreading margins of altered density (Fig. 4). However, not all combinations of *Y. lipolytica* and *D. hansenii* gave this phenomenon, suggesting that it was strain-related.

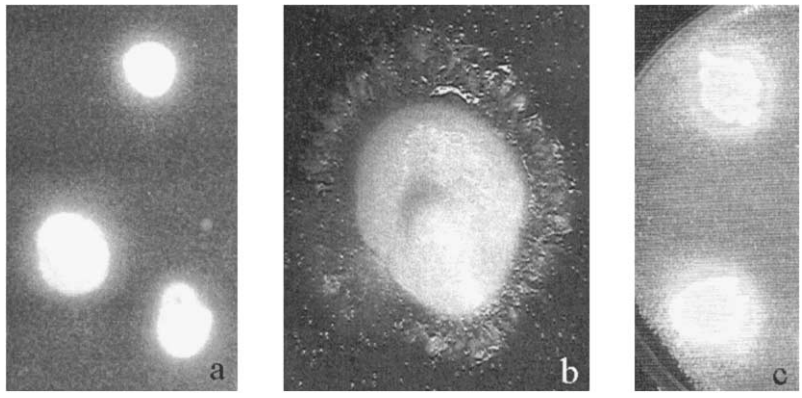

Fig. 4. Microbial interactions on "spot-on-lawn" plates. (a) *D. hansenii* (spotted strains) enhances the growth of *Y. lipolytica*. (b) Unusual colony morphology of *Y. lipolytica* growing on a lawn of *D. hansenii*. (c) *D. hansenii* (spotted strains) enhances the growth of *S. xylosus*.

3.4.2. Yeast–bacteria interactions

As determined by the spot-on-lawn assay, none of the strains of *D. hansenii*, *Y. lipolytica*, *K. marxianus* and *Sac. cerevisiae* inhibited any of the isolates of *Staphylococcus* spp. (15 strains) or *Micrococcus* spp. (four strains) obtained from cheeses A, B or C. Similarly, they were not inhibitory to strains of *E. faecium* or *E. faecalis* that were isolated from these cheeses on other occasions. None of the bacteria were inhibitory to the yeasts. However, two strains of *D. hansenii* enhanced the growth of isolates of *S. xylosus* (Fig. 4) and four strains enhanced the growth of *E. faecium* and *E. faecalis*. One strain of *Y. lipolytica* enhanced the growth of *S. xylosus*. The growth of *Enterococcus* spp. was also enhanced by strains of *Y. lipolytica*, *K. marxianus* and *Sac. cerevisiae*.

Various strains of *D. hansenii*, *Y. lipolytica*, *K. marxianus* and *Sac. cerevisiae* did not exhibit antagonistic activity against strains of the pathogens, *Sal. typhimurium*, *Escherichia coli* or *S. aureus*, but some strains of *Y. lipolytica* gave weak inhibition of *L. monocytogenes* and *B. cereus*.

4. Discussion

To better understand the significance of microorganisms in cheese production, we have studied the growth profiles of yeast and bacteria in two Camembert and two blue-veined cheeses produced in commercial operations in Australia. Our findings confirm and extend knowledge on this topic.

In agreement with cheeses produced in other countries, yeasts were prominent in the maturation process, with *D. hansenii* being the principal species which grew (Fleet, 1990; Jakobsen and Narvhus, 1996). Generally, it grew to maximum populations of 10^8–10^9 cfu/g within the first week of maturation and remained at this level throughout the process. However, in one process (Camembert A), it reached a maximum population of only 10^5–10^6 cfu/g. This difference raises the question as to which factors affect or limit the growth of this species during cheese maturation. Concentration of NaCl, temperature, oxygen availability, substrate availability (e.g. lactate), and influences of other microorganisms are all possible factors that require systematic examination—especially if it is considered that the growth of this species contributes positively to cheese quality (Leclercq-Perlat et al., 1999; Martin et al., 1999; Wyder and Puhan, 1999). Previous surveys of retail cheeses in Australia demonstrated the high incidence and populations of this species, but there were many instances where it was not found in cheeses (Roostita and Fleet, 1996a). To the best of our knowledge, the cheeses we examined were not inoculated with this yeast, which developed as a natural contaminant of the process. We frequently isolated *D. hansenii* from brine tanks, which would be the primary source of the yeast because of its tolerance of high concentrations of NaCl. Man-

agement of the brine used by cheese manufacturers is likely to be a significant factor that affects the occurrence of yeasts in cheese maturation. The number of times the brine is reused in cheese processing, as well as its pasteurisation, filtration or centrifugation would be important variables.

Y. lipolytica was less prominent in maturation of the cheeses (Figs. 1–3) than might have been expected from the data of previous surveys (Roostita and Fleet, 1996a) and other reports (Guerzoni et al., 1998; Freitas et al., 1999). It did not reach the high populations that were found for *D. hansenii*, and it tended to develop later in the maturation process. Although some isolates of *D. hansenii* from cheese were positive for the killer effect, we found no evidence of their antagonism towards *Y. lipolytica*. The presence of NaCl in the environment has been reported to increase the susceptibility of yeasts to the killer effect and the diversity of yeasts species that are affected (Llorente et al., 1997). However, none of the strains of *Y. lipolytica* examined in our study became sensitive to killer strains of *D. hansenii* when NaCl was incorporated into the assay medium. Interestingly, we found some evidence that *D. hansenii* could enhance the growth of *Y. lipolytica* (Fig. 4) and this observation is worthy of further investigation. *Y. lipolytica* is a strong producer of proteolytic and lipolytic activities (Table 1) (Roostita and Fleet, 1996b), making it an attractive candidate to develop as a commercial starter culture to accelerate the maturation process (Guerzoni et al., 1998). For this initiative to progress, more information is needed about the factors which determine its growth and activity during cheese maturation. Careful strain selection will be important for its use as a starter culture since some strains might negatively impact on cheese quality (Valdes-Stauber et al., 1997; Wyder and Puhan, 1999).

The potential for positive or negative yeast–bacterial interactions to occur during cheese maturation has been reported previously (Fleet, 1990) and was the basis for concurrently monitoring the bacterial populations in the cheeses with the yeasts (Figs. 1–3). Moreover, the growth profiles of bacterial species throughout cheese maturation, generally, are not well-known. Our findings support previous conclusions that the growth of staphylococci and micrococci can be significant during maturation (Choisy et al., 1987; Bhowmik and Marth, 1990; Vivier et al., 1994; Stanley, 1998a,b). The principal species identified in our study were *S. xylosus* and *M. varians*. Many of these isolates were significant for protease and lipase production (Table 1), indicating their potential impact on cheese flavour and texture. The growth profiles of these bacteria varied with the cheese and further study is needed to determine which factors are important in affecting this growth. Interestingly, growth of these bacteria occurred in the later stages of maturation, after yeast growth. The utilisation of lactic acid by yeasts and the associated increase in pH (Figs. 1–3) is one factor that could contribute to this sequential development (Fleet, 1990). However, the yeasts may enhance bacterial growth by other mechanisms, as suggested by the observations in Fig. 4. Further studies to determine the basis of these growth-promoting interactions are needed.

The significant growth of *Acinetobacter* species during the maturation of both Camembert cheeses A and B was an unexpected observation and appears to be the first report of the association of these species with this process. Approximately half of the isolates obtained were lipolytic (Table 1) and they can utilise lactate (Holt et al., 1994). These two properties could explain their ability to grow in the cheese ecosystem. Their absence from blue-veined cheeses, but not Camembert cheese, might be related to an inability to tolerate the higher salt contents of the blue-veined varieties. *Acinetobacter* species are ubiquitous in soil, water and food, especially meats, poultry and seafood, but they have also been reported in milk and cottage cheese (Kampfler, 1999). It needs to be determined how these bacteria impact on cheese quality during maturation, but with meat products, they are considered to be spoilage organisms. Some species of *Acinetobacter* degrade mycotoxins, which could be a useful property (Hwang and Draughon, 1994).

In surveys of retail samples of Camembert and blue-veined cheeses produced in Australia, we have frequently found them to contain significant populations ($> 10^3$–10^4 cfu/g) of other bacteria including species of *Enterococcus*, *Propionibacterium*, *Brevibacterium*, *Bacillus*, *Lactobacillus* and *Enterobacteriaceae* (unpublished data). However, plating of cheese samples on a range of selective media was

necessary to isolate these groups of bacteria. These findings suggest that the microbial ecology of maturation is probably far more complex than that found in the present investigation. More detailed monitoring of the maturation process using a battery of selective–differential media to isolate target groups of bacteria and yeasts will be needed to reveal this complexity. In addition, it is unlikely that traditional, cultural methods will be adequate to reveal the complete ecology and that molecular approaches based on the isolation and study of microbial DNA will be necessary to advance the field (Muyzer and Smalla, 1998; Fleet, 1999).

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