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Growth and survival of *Bacillus cereus* in mageu, a sour maize beverage

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Abstract

The growth and survival of *Bacillus cereus*, a known pathogen commonly found in cereals, during lactic acid fermentation of mageu, a sour maize beverage, was studied. In the mageu base inoculated with both the starter culture and *B. cereus,* the acidity developed to pH ≤ 4.00 and 0.10% titratable acidity after 24 h; the growth of *B. cereus* was reduced from 10^6 c.f.u./ml to 10^2 c.f.u./ml within 24 h; after the first 6 h of fermentation, the rate of inhibition of *B. cereus* was correlated to the rate of decrease in pH ($r = 0.85$, $p < 0.05$); the redox potential (E_h) decreased from 463 to 149 mV within the first 12 h. The control mageu base to which neither starter nor lactic acid was added, had a pH of 6.50, titratable acidity of 0.015% and lowest E_h of 244 mV. In the mageu base to which lactic acid and *B. cereus* were added, the pathogen was inhibited to $< 10^1$ c.f.u./ml. The *B. cereus* in the mageu base to which no starter culture nor lactic acid was added, grew to over 10^7 c.f.u./ml after 12 h. The decrease in E_h seemed to have no inhibitory effect on the growth and survival of *B. cereus*. No strains of lactic acid bacteria were found to produce bacteriocins antagonistic to *B. cereus*. Low pH and acidity were found to be the major factors inhibiting growth of *B. cereus* in mageu.

Introduction

Lactic acid fermentation of foods is widely practised in Southern African countries as a household-level technology to process and preserve a number of products. Mageu is a sour, non-alcoholic beverage popular among the indigenous people of Southern Africa, including farmers, school children and mine workers. It is consumed as a refreshing drink as well as a weaning food. Mageu is prepared by boiling maize flour in water to make a porridge of 8 to 10% solids (w/w) to which wheat flour is added to initiate the lactic acid fermentation. The traditional processing and properties of mageu have been reviewed by Van Noort & Spence (1976), Hesseltine (1979), Tomkins *et al.* (1987) and Holzapfel (1989).

Foodborne illnesses are a health problem in developing countries particularly among infants and children. It is estimated that about 70% of the episodes of diarrhoeal diseases are foodborne in origin, resulting from food contaminated by dirty utensils, poor processing, handling and storage conditions (Motarjemi & Nout 1996). *Bacillus cereus* is a known spore forming pathogen (Johnson 1984) and has been found in dried foods such as pulses and cereals (Blakey & Priest 1980; Rusul & Yaacob 1995). It is possible that maize flour and other cereals used in the making of mageu can be contaminated with *B. cereus*. Although cooking of the porridge inactivates most contaminating micro-organisms, heat resistant bacterial endospores may survive or even be stimulated to germinate (Davies & Wilkinson 1973). Recontamination of the cooked porridge may occur through handling, utensils and the addition of wheat flour (Nout *et al.* 1987b). This may lead to microbial growth and possibly prove to be a health hazard depending on the nature and extent of the contamination as well as the storage conditions. The effects of the resulting hazard can be more serious among infants when mageu is used as a weaning food. Such recontamination is important to mageu because it is consumed without any heat treatment after fermentation (Holzapfel 1989).

It has been shown that lactic acid fermented foods with pH ≤ 4.0 inhibit the growth of *B. cereus* and other pathogens (Nout *et al.* 1987a; Aryanta *et al.* 1991; Svanberg *et al.* 1992; Kingamukono *et al.* 1994). The different ways in which lactic acid bacteria inhibit pathogens have been reviewed by Lindgren & Dobrogosz (1990). Most of the work done on the inhibition of pathogens in lactic acid fermented foods has concentrated on the effect of pH and acidity, however, it is possible that apart from the low pH and acidity, other inhibitory factors like the production of bacteriocins (Olsen *et al.* 1995) and lowering of the E_h (Tomkins *et al.*

1987) by lactic acid bacteria could be involved in the inhibition of *B. cereus*.

The aim of this study was to determine the role of lactic acid bacteria in the inhibition of *B. cereus* in mageu produced by the recycling technique and to evaluate the effect of acidity, low pH, E_h and the possibility that the lactic acid bacteria involved in mageu fermentation may produce bacteriocins antagonistic to *B. cereus*.

Materials and Methods

Samples and preparation of mageu base

Maize flour, wheat flour and cane sugar were purchased from a supermarket. A slurry containing 8% (w/w) maize flour, 91% (w/w) water and 1% (w/w) cane sugar was prepared. The slurry was cooked for 30 min to make 400 ml of a porridge that was divided into two equal parts and cooled to 35 °C. This porridge was referred to as mageu base.

Starter culture preparation

To start the fermentation, wheat flour was added to each of the two batches of mageu base at a rate of 5% (w/w) of the maize flour. The mixture was thoroughly stirred and fermented at 35 °C for 24 h to yield duplicate batches of preliminary mageu culture. The preliminary mageu cultures were recycled in mageu base at an inoculation rate of 5% (v/v) followed by incubation at 35 °C for 24 h. This procedure was repeated three times leading to duplicate batches of mageu culture, hereafter referred to as starter cultures.

Experimental design

Five duplicate samples, each consisting of 500 ml of sterile mageu base (121 °C, 15 min), were prepared. The first batch of mageu base was inoculated with 5% (v/v) starter culture and approximately 10^3 c.f.u./ml *B. cereus* ATCC 11778. A second batch of mageu base was inoculated with approximately 10^3 c.f.u./ml *B. cereus* ATCC 11778 only (no starter culture). A third batch of mageu base was artificially acidified by the addition of lactic acid to adjust the pH to between 3.80 and 4.00 and then inoculated with approximately 10^3 c.f.u./ml of *B. cereus* ATCC 11778. The fourth batch of mageu base was not inoculated and served as the control.

All the sample treatments were thoroughly mixed and incubated at 35 °C for 24 h. At 6 h intervals, the pH, titratable acidity, E_h, and the number of viable *B. cereus* and lactic acid bacteria were determined in each sample. The titratable acidity was determined by titrating 10 g of sample, diluted with 10 ml distilled water, with 0.11 M NaOH and phenolphthalein as an indicator. The acidity was calculated as per cent lactic acid (w/w) (Ayebo & Mutasa 1987). The pH was measured with a glass electrode connected to a standard pH-meter PHM82 (Radiometer, Copenhagen, Denmark). The E_h was measured using a platinum electrode, M21Pt and a saturated calomel reference electrode, K401 (Radiometer, Copenhagen). The two electrodes were connected to a standard pH meter, PHM82 and the readings recorded in millivolt. The E_h of the sample treatments at pH 7.0 was calculated using the formula adapted from Rodel & Lucke (1990).

Bacterial strains

B. cereus ATCC 10702 and *B. cereus* ATCC 11778 were obtained from the South African Bureau of Standards (Pretoria) and *Listeria monocytogenes* B88 and *Lactococcus lactis* ATCC 11454 from the culture collection of the Foodtek Division of the Council for Scientific and Industrial Research (Pretoria). Before use, strains of *B. cereus* and *L. monocytogenes* were separately grown overnight at 30 °C in Tryptone soya broth (Oxoid CM129) and *L. lactis* ATCC 11454 in MRS broth (Oxoid CM359).

Bacterial techniques

Viable colonies were counted by spread plating 0.1 ml of serial dilutions of the samples onto sterile *B. cereus* selective medium (Oxoid CM617) for *B. cereus* and MRS agar (Oxoid CM361) for the lactic acid bacteria. The *B. cereus* selective medium plates were incubated aerobically at 35 °C for 24 h while the MRS agar plates were incubated anaerobically using Anaerocult A (Merck, Darmstadt, Germany) at 35 °C for 48 h. The numbers of c.f.u. per ml of sample were calculated after incubation according to Anon. (1987).

Isolation and characterization of lactic acid bacteria

Lactic acid bacteria were isolated from the mageu starter culture using the spread plate technique. The selected colonies were subcultured in MRS broth and streaked onto MRS agar to obtain pure cultures which were examined for cell morphology, Gram reaction and catalase reaction.

Bacteriocin assay

A modification of the spot-on-the-lawn method (Van Belkum *et al.* 1989) was used to screen 100 lactic acid bacteria strains isolated from the starter culture for bacteriocin production. Using a multi-point inoculator (Mars Laboratories, Liverpool, England), lactic acid bacteria were replica-spot inoculated onto Brain Heart Infusion (Oxoid CM225) with 1.4% agar as the base medium. The plates were then incubated anaerobically at 30 °C for 24 h. Tryptone soya broth (7 ml) containing 0.7% agar, tempered at 45 °C and seeded with 100 μl each of, respectively, *B. cereus* ATCC 11778, *B. cereus* ATCC 10702 and *L. monocytogenes* B88, was used as the

overlay medium. The overlaid plates were incubated aerobically overnight at 30 °C. Inhibition was detected by a zone of clearing around the producer colony. The diameter (mm) of the zone of inhibition was measured.

To confirm that a bacteriocin-like substance was produced, proteinase-K and pepsin (Boehringer Mannheim, GmbH Germany), each at 20 mg/ml, were filled in holes made in the agar next to the lactic acid bacteria colonies and the plates incubated at 30 °C for 3 h before they were overlaid. Plates were examined to judge whether the inhibitory substance was sensitive to proteolysis. Bacterial isolates that produced a bacteriocin-like inhibitory substance were identified to species level using API 50CHL kits (bioMerieux, Missouri, USA).

Statistical analysis

Microbial counts were converted to $\log_{10}$ c.f.u./ml and subjected to ANOVA using Statistica Version 5 (StatSoft, Inc., Tulsa OK, USA). Correlations, means, standard deviations and the least significant difference between the means were determined ($p < 0.05$).

Results

Figure 1 and Table 1 show a general decrease in pH and an increase in titratable acidity of the mageu base inoculated with *B. cereus* and the starter culture during the 24 h fermentation at 35 °C. A decrease in the E_h and *B. cereus* numbers was observed while the lactic acid bacteria increased. After the first 6 h, the rate of decrease in the number of viable *B. cereus* was positively correlated ($r = 0.85, p < 0.05$) with the rate of decrease in pH. In the mageu base inoculated with *B. cereus* alone, the pH and E_h decreased as the number of viable *B. cereus* increased (Figure 2). In the mageu base acidified by the addition of lactic acid and inoculated with *B. cereus*, the titratable acidity (results not shown) and pH remained fairly steady while the number of viable *B. cereus* and the E_h decreased (Figure 3). The control mageu base (Figure 4) showed a variation in the E_h while the pH and titratable acidity (results not shown) remained steady; no lactic acid bacteria or *B. cereus* were detected in the control during the 24 h. No strains of lactic acid bacteria isolated from the starter culture produced bacteriocins antagonistic to *B. cereus*. However, isolates YM36 and YM37 from the starter culture,

Table 1. Changes in the mean titratable acidity (% lactic acid w/w) of mageu base inoculated with *B. cereus* and the starter culture during 24 h incubation at 35 °C.

	Time (h)				
	0	6	12	18	24
Titratable acidity	0.020a[†]	0.030ab	0.060abc	0.085bc	0.095c
	(0.000)[‡]	(0.014)	(0.014)	(0.007)	(0.007)

[†] Means with different letters are significantly different ($p < 0.05$).
[‡] Numbers in parentheses are standard deviation.

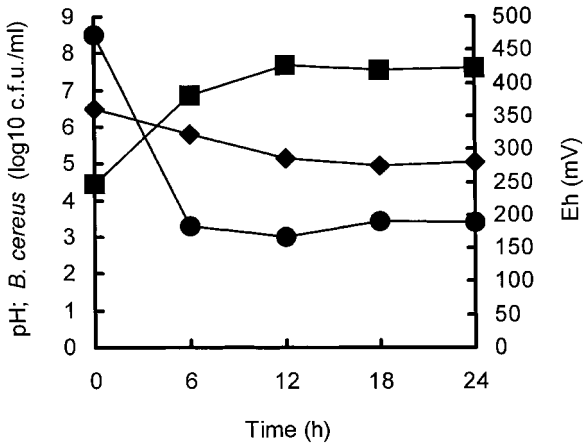

Figure 2. Changes in pH (◆), redox potential (●) and *B. cereus* (■) in mageu base inoculated with *B. cereus* alone during 24 h incubation at 35 °C.

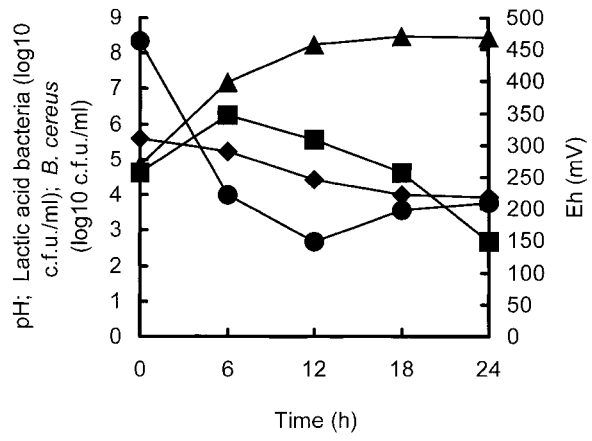

Figure 1. Changes in pH (◆), redox potential (●), lactic acid bacteria (▲) and *B. cereus* (■) in mageu base inoculated with the starter culture and *B. cereus* during 24 h fermentation at 35 °C.

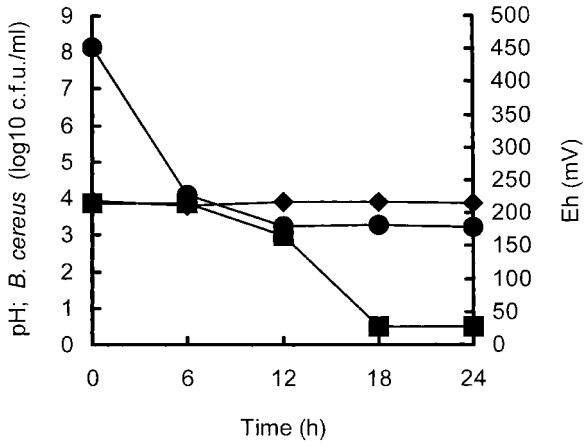

Figure 3. Changes in pH (◆), redox potential (●) and *B. cereus* (■) in mageu base acidified by addition of lactic acid and inoculated with *B. cereus* during 24 h incubation at 35 °C.

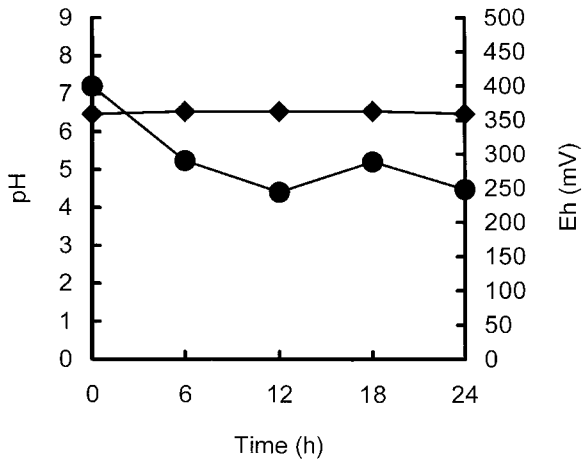

Figure 4. Changes in pH (◆) and redox potential (●) of the control mageu base during 24 h incubation at 35 °C.

identified as strains of *L. lactis*, exhibited bacteriocin-like activity against *L. monocytogenes* (Table 2).

Discussion

B. cereus was able to grow to more than 10^7 c.f.u./ml in the mageu base which was not fermented by lactic acid bacteria. A similar trend was reported in unfermented cereal gruels (Svanberg *et al.* 1992) and in unfermented soybeans meant for making tempe (Nout *et al.* 1987a). This implies that, on its own, *B. cereus* could grow in maize porridge to potentially toxic levels. However, in a lactic acid fermenting product like mageu, *B. cereus* was reduced to smaller numbers within 24 h. Similar inhibition of *B. cereus* by lactic acid bacteria in naturally fermenting foods with pH ≤ 4.00 was reported by Aryanta *et al.* (1991), Svanberg *et al.* (1992) and Kingamukono *et al.* (1994). Inhibition of pathogens by lactic acid bacteria is important for mageu preparation because its fermentation process is started by the addition of wheat flour and the fermented product is not subjected to a heat treatment before consumption. The wheat flour and utensils used in the preparation of mageu are potential sources of contamination with *B. cereus*. The inhibition of *B. cereus* was also achieved in the artificially acidified mageu base where the average pH and titratable acidity remained fairly constant. Svanberg *et al.* (1992) reported similar inhibition of *B. cereus* in acidified gruel. Thus, the decrease in the viable *B. cereus* was possibly due to the low pH and high acidity resulting from the added lactic acid.

In the mageu base inoculated with *B. cereus* and the starter culture, *B. cereus* increased by two log cycles in the first 6 h, probably because the pH was still above 5.0. A decrease in the number of viable *B. cereus* was observed after the first 6 h when the pH fell below 5.0. According to Goepfert *et al.* (1972) and Nout *et al.* (1987a), the minimum pH for *B. cereus* to grow is about 4.90. Considering the correlation between the rate of decrease of *B. cereus* and that of pH after the first 6 h, the decrease in *B. cereus* between 6 h and 18 h could be attributed mainly to the decrease in pH. Between 18 and 24 h, a statistically insignificant change in pH was accompanied by approximately 2 log cycles decrease in the number of viable *B. cereus*. This could possibly be attributed to the inhibitory effect of the undissociated acid molecules. According to Nout & Rombouts (1992), the inhibitory effect of undissociated organic acid molecules is 10–600 times stronger than that of their dissociated forms.

The E_h values observed in the mageu base inoculated with *B. cereus* and the starter culture; the mageu base inoculated with *B. cereus* alone; and the acidified mageu base inoculated with *B. cereus* ranged between 149 mV and 473 mV. However, over this range of E_h, the number of viable *B. cereus* decreased in the mageu base inoculated with the starter culture and in the acidified mageu base but increased in the mageu base inoculated with *B. cereus* alone. Thus, the decrease in viable *B. cereus* observed in the acidified mageu base and the mageu base inoculated with the starter culture was perhaps not due to the observed decrease in E_h. Rodel & Lucke (1990) found that the E_h values in pasteurised meat products (range between +250 and −150 mV) had no effect on the growth of *Bacillus licheniformis*. The E_h values encountered in this work were in the oxidized range (Anon. 1980) where most aerobic bacteria are able to grow and metabolize. *B. cereus*, like *B. licheniformis*, is a facultative anaerobe (Johnson 1984), able to grow over E_h values in both the reduced and oxidized ranges.

This study showed that, given the right conditions, *B. cereus* can grow to potentially toxic levels in unfermented maize porridge. Such a scenario can be exhibited when unfermented porridge is stored at ambient tem-

Table 2. Inhibition of strains of *B. cereus* and *L. monocytogenes* by different lactic acid bacteria where the inhibitory substance was sensitive to protease action.

Indicator organism	Diameter of zone of inhibition (mm)		
	L. lactis 11454[†]	*L. lactis* YM36[‡]	*L. lactis* YM37[‡]
B. cereus 11778	0.0	0.0	0.0
B. cereus 10702	1.5	0.0	0.0
L. monocytogenes B88	5.0	5.0	5.0

[†] A known bacteriocin producer used as positive control.
[‡] Strains isolated from the mageu starter culture.

perature for long periods, a practice that is common in poor households in developing countries. However, the fermentation process of mageu inhibited the pathogen to low numbers within 24 h. Therefore, lactic acid fermentation can reduce the health risk posed by pathogens like *B. cereus*.

The lactic acid bacteria involved in mageu fermentation did not produce bacteriocins antagonistic to *B. cereus*. Although the E_h decreases during mageu fermentation, the values achieved seemed not to inhibit the growth and survival of *B. cereus* in this product. The production of lactic acid and its pH-lowering effect were the major inhibitory factors. Thus, lactic acid production by the starter culture should be considered a critical factor in ensuring the safety of mageu. Lactic acid production should, therefore, be maximized by optimizing fermentation conditions and, where possible, by careful selection and development of fast acid-producing starters.

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