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Short communication

Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic *Escherichia coli* and *Salmonella enteritidis* strains

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Abstract

The survival and growth of *Escherichia coli* 3339 and *Salmonella enteritidis* 949575 isolated from human clinical samples, in milk fermented with lactic acid bacteria (LAB) and yeast strains previously isolated from Zimbabwean naturally fermented milk (NFM) was studied. The LAB starter cultures used were *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* C1 alone (C1) or in combination with *Candida kefyr* 23 (C1/23), *L. lactis* subsp. *lactis* Lc261 alone (LC261) or in combination with *C. kefyr* 23 (Lc261/23). The growth of the same pathogens in milk fermented with a commercial DL culture (CH-N 22) and spontaneously fermented raw milk was also monitored. The C1 and C1/23 cultures significantly ($P<0.05$) inhibited the growth of both pathogens. When inoculated at the beginning of the fermentation, both *E. coli* 3339 and *S. enteritidis* 949575 counts were significantly ($P<0.05$) reduced by about two log cycles in C1 and C1/23 cultured milk. However, in naturally fermented milk and the DL cultured milk, both *E. coli* 3339 and *S. enteritidis* 949575 grew and reached high populations of about 9 and 8.8 log cfu ml^{-1}, respectively, after 18 h. When *E. coli* 3339 was inoculated into previously fermented milk, the viable counts were significantly ($P<0.05$) reduced in the presence of C1 and C1/23 from 7 log cfu ml^{-1} to 3 log cfu ml^{-1} after 48 h. *S. enteritidis* 949575 could not be recovered from these cultures after 48 h. The addition of the yeast did not enhance or diminish the inhibitory capacity of the LAB cultures. The pathogens survived in high numbers when inoculated into pre-fermented NFM and the commercial DL- (CH-N 22) cultured milk. The C1 strain, therefore, offered the best protection against the pathogens. Its inhibitory effect was mainly related to fast acid production.

Keywords: Naturally fermented milk; Lactic acid bacteria; Yeast; Pathogens

1. Introduction

Fermented foods are consumed widely worldwide (IDF, 1988). In Zimbabwe, both naturally fermented milk (NFM) (also known as *amasi*) and commercially produced cultured milks are popular with consumers. The NFM is consumed as a weaning food, a refreshing drink or with other food products (Mutukumira, 1995). Consumption of fermented foods has many advantages including enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens.

In Zimbabwe, NFM is produced by allowing raw milk to spontaneously ferment at ambient temperatures of around 25 °C. A variety of microorganisms including lactic acid bacteria, yeast moulds and coliforms have been isolated from NFM (Mutukumira, 1996; Gadaga et al., 2000; Gran et al., 2003a). The fermentation process and product quality vary depending on the predominant microorganisms in the milk. Despite the inconsistent quality of the product, naturally fermented milk is apparently considered to be more organoleptically superior to similar commercially produced cultured products in Zimbabwe (Feresu and Muzondo, 1989; Mutukumira, 1996). The difference between the two types of milks has been attributed to the types of microorganisms which produce different types of flavor compounds (Gadaga et al., 2001; Gran et al., 2003b). It

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has therefore, become obvious that there is need to develop starter cultures that can be used to produce safe products with reproducible quality and capture the desirable attributes of NFM.

Fermented food products are usually considered safe because of the low pH and production of antimicrobial substances by fermenting organisms. However, some pathogens such as *Escherichia coli* 0157:H7, *Listeria monocytogenes* and *Salmonella enteritidis* have been reported to survive and grow in fermented milks (Feresu and Nyathi, 1990; Farber and Peterkin, 1991). This is of concern because these foods are also used as weaning foods. Mortality and morbidity rates due to diarrhoeal diseases are highest in infants during the weaning period (Nout et al., 1989). The safety of fermented milk products therefore becomes a major public health issue.

During fermentation of milk, the main metabolic product of LAB fermentation is lactic acid. The other microbial groups such as yeasts could contribute to the overall characteristics of the fermented milk, especially the flavour profile. Some lactic acid bacteria strains isolated from Zimbabwean naturally fermented milk have been shown to produce fermented milk products that are acceptable and may be commercially viable (Mutukumira, 1996). However the safety of these cultures has not been studied. Use of starter cultures with the potential to produce inhibitory factors would result in the improved safety of fermented products (Olsen, 1990; Russell, 1982). The objective of the present study was to assess the safety of the fermented milk products produced using pure lactic acid bacteria cultures and a combination of lactic acid bacteria and yeast isolated from Zimbabwean traditional fermented milk by monitoring the survival of pathogenic *E. coli* and *S. enteritidis* during fermentation. The survival of these pathogens in commercial DL-cultured milk and spontaneously fermented milk was also studied.

2. Materials and methods

2.1. Materials

Raw milk was collected from a farm near Harare and used within 3 h of collection. Ultra high temperature (UHT)-treated milk (Dairibord, Harare, Zimbabwe) was bought from a local supermarket in 500 ml Tetrapak cartons. A pure culture of *Candida kefyr* (23) originally isolated from Zimbabwean traditional fermented milk was obtained frozen from The Institute of Food Nutrition and Family Sciences (IFNFS), University of Zimbabwe. It was resuscitated and stored on Yeast extract-Malt extract (YM) (Merck, Darmstadt, Germany) agar slants at 5 °C.

Lactic acid bacteria (LAB) strains, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* C1 and *L. lactis* subsp. *lactis* Lc261 were also obtained from the IFNFS. The C1 strain was obtained as a freeze dried culture, while Lc261 was a frozen culture. The DL culture was obtained from Christian Hansen (CH-N 22, Hørsholm, Denmark) as a freeze-dried culture.

The pathogens, *E. coli* 3339 and *S. enteritidis* 949575, previously isolated from patients suffering from food poisoning (diarrhoea), were obtained from CIMAS Medical Chambers Bacteriology Laboratories, Harare, Zimbabwe. The pathogens were maintained on MacKonkey agar plates (Oxoid, Basingstoke, UK) at 5 °C.

2.2. Resuscitation of cultures

The concentrated yeast culture was thawed and inoculated (0.1%) into sterile Yeast Extract-Malt Extract (YM) (Merck) broth (10 ml), and incubated at 25 °C for 48 h. The yeast was further sub-cultured by inoculating (0.1%) into sterile 10% (w/v) reconstituted skim milk (Oxoid) and incubating at 25 °C for 48 h.

Portions (ca. 0.1 g) of each of the freeze dried *L. lactis* subsp. *lactis* biovar. *diacetylactis* C1 and DL-culture were aseptically suspended into MRS (Merck) broth (10 ml) and incubated at 30 °C for 24 h. Similarly, a small portion (1 ml) of the thawed Lc261 culture was added to another tube containing MRS (Merck) broth (10 ml) and incubated for 24 h at 30 °C. The revived cultures (1%) were inoculated separately into sterile reconstituted (10%, w/v) skimmed milk (Oxoid) (10 ml) and incubated at 30 °C for 18 h prior to use.

The pathogens were revived by picking a loopful of each of pathogenic *E. coli* 3339 and *S. enteritidis* 949575 from the MacKonkey agar (Oxoid) plates and separately inoculating into sterile Nutrient broth (Merck) (10 ml). This was further transferred (5%) into sterile reconstituted skimmed milk (Oxoid) (10%, w/v) (10 ml) and incubated at 37 °C for 24 h.

2.3. Fermentation

A portion of UHT milk (Dairibord) (50 ml) was aseptically transferred into 100 ml sterile screw-capped bottles and steamed for 10 min. The steamed milk samples were cooled to ambient temperature and inoculated using actively growing cultures of lactic acid bacteria (1%) and yeast (0.1%), singly or in combination. The single and paired combinations of the starter strains that were used were:

a. *L. lactis* subsp. *lactis* biovar. *diacetylactis* C1 alone.
b. A combination of *L. lactis* subsp. biovar. *lactis* diacetylactis and *C. kefyr* 23, (C1/Y23).
c. *L. lactis* subsp. *lactis* Lc261 (Lc261), alone.
d. A combination of *L. lactis* subsp. *lactis* Lc261 and *C. kefyr* 23 (Lc261/Y23).

Actively growing cultures of the pathogens were then added separately into the inoculated milk to give initial approximate pathogen counts of about 7 $\log_{10}$ cfu ml^{-1}. The samples were incubated at 25 °C for 48 h. The control was UHT milk inoculated with either *E. coli* 3339 or *S. enteritidis* only.

The growth and survival of the microorganisms were monitored by drawing samples after 0, 6, 12, 18, 24, and 48 h. Viable counts of *E. coli* 3339, *S. enteritidis* 949575, *C. kefyr* 23 and lactic acid bacteria were determined, together with the pH and titratable acidity.