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# LACTIC ACID BACTERIA ISOLATES FROM FERMENTED CAMEL MILK (*SUUSAC*) ARE POTENTIAL PROTECTIVE CULTURES OF RAW CAMEL MEAT

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## ABSTRACT

Camel meat is the mainstay for the inhabitants of arid lands due the resilience and adaptation of camel. However, the lack of sufficient information regarding microbial stability and safety hinders utilisation and market competitiveness of the meat. This study, therefore, aimed at characterising the potential of Lactic Acid Bacteria (LAB) isolates adapted to camel meat production environment as potential protective cultures against Enterobacteriaceae-the most challenging bacterial contaminant of raw meat- present on raw camel meat. Seven LAB and ten members of *Enterobacteriaceae* were isolated and characterised from *suusac* and raw camel meat, respectively. The antimicrobial activity of the LAB against the *Enterobacteriaceae* members was evaluated by the agar well diffusion assay. *Citrobacter* spp., *Shigella* spp. and three out of the seven *E. coli* isolates were inhibited, while *Salmonella* spp. was not inhibited by the LAB isolates. The mean diameters of the zone of inhibition ranged from 8.5 mm to 12.5 mm. There was no significant difference between the mean diameters of inhibition zone among the inhibited *Enterobacteriaceae* members ($P>0.05$). This study established that raw camel meat may harbour foodborne pathogenic *Enterobacteriaceae* of a serious concern. LAB from *suusac*, on the other hand, may repress the growth of some of them through antagonistic interactions. Therefore, LAB showed potential as protective cultures in improving safety and quality of raw camel meat.

**Keywords:** Lactic acid bacteria, Fermented camel milk, Raw camel meat

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## INTRODUCTION

Despite of being an important member of the food-producing animals, the camel has over the years been the most neglected animal in terms of its improvement and scientific research compared to other animals (Kalalou et al., 2010; Skidmore, 2006). Furthermore, the lack of interventions regarding the microbial stability and safety of the raw meat has considerably reduced its market potential (Mahmud et al., 2011). Traditional preservation techniques, for instance, processing into *tichtar* (in Morocco) and *nyirinyiri* (in Kenya) through sun drying have several health risks to the consumers. The sun drying process involves the exposure of meat surface to both physical and microbial contaminants (Food and Agriculture Organization, 2007). In fact, a recent study established the occurrence of a high microbial contamination in *nyirinyiri* (Kisembe et al., 2015). On the other hand, modern preservation techniques, such as addition of chemical preservatives, are unacceptable to many consumers (Cudjoe and Kapperud, 1991; Hugas, 1998; Stiles, 1996); and, as an alternative, the use of refrigeration in the Arid and Semi-Arid Lands (ASALs) is not technically and economically feasible.

In an attempt to harmonise the consumer demands with the necessary safety standards, the combinations of innovative technologies that include biological antimicrobial systems such as Lactic Acid Bacteria (LAB) (Ananou et al., 2007) are replacing the traditional means of controlling microbial safety hazards in foods. The use of LAB is not new; they have been in use for centuries to preserve food (Einarsson and Lauzon, 1995). In this respect, fermented and cooked meat preservation by LAB has been a common practice for many years. A variety of strains has been reported to be antagonistic to pathogenic and spoilage microorganisms associated with these products (Laursen et al., 2005). The antagonistic properties of such microorganisms are conferred by the production of one or more antimicrobial metabolites such as organic acids (lactic and acetic), hydrogen peroxide, antimicrobial peptides (bacteriocins) and bioactive enzymes (Kalalou et al., 2004; Millette et al., 2007). In this regard, several studies have been carried out but very few are focused on preservation of raw red meat using these bactericidal microorganisms (Laursen et al., 2005).

Some recent studies have shown the preservative potential of LAB in processed meat products against members of the *Enterobacteriaceae* family as well as other microbial contaminants (Al-Allaf et al., 2009; Balia et al., 2009; Bredholt et al., 1999, 2001; Héquet et al., 2009; Jones et al., 2008; Kotzekidou and Bloukas, 1996; Pidcock et al., 2002). This is a significant breakthrough given that *Enterobacteriaceae* has an epidemiological potential and importance, as some of its members are pathogenic and may cause serious infections and/or food poisoning. In addition, they also initiate spoilage in meat by raising its pH (Al-Mutairi, 2011; Pal et al., 2005). In order to develop preservation and safety methods for raw meat, promising strains suitable for selection

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and implementation as protective cultures need to be identified and used to improve the safety and quality of the meat (Jones et al., 2008; Pal et al., 2005). It has been suggested, therefore, that the application of protective cultures to raw meat should be considered as an additional safety factor in addition to other preservation methods (Signorini et al., 2006).

There has been no study so far considering the potential of LAB to stabilise the microbiological safety of raw camel meat. Only two studies are related, but they focused on the extension of the shelf life of camel meat products, specifically minced camel meat through fermentation (Kalalou et al., 2004) and the use of acidic organic salts combined with Bifidobacterium (Al-Sheddy et al., 1999). Therefore, this study aimed at characterising the potential of LAB isolates from *suusac* (fermented camel milk), which were adapted to the camel production environment as potential protective cultures in raw camel meat. It is expected that these LAB would be promising as protective cultures of raw camel meat; and it would be feasible to enhance the safety and quality of preserved camel meat in such a way.

## MATERIALS AND METHODS

### Sample acquisition, transport and preparation

Samples of raw camel meat and *suusac* originating from Isiolo—a semi-arid high camel production region—were obtained in Eastleigh in Nairobi City, Kenya. The meat samples were obtained from purposively sampled butcheries by cutting off meat with a sterile knife, and placed separately in sterile polythene bags. One carcass per butchery was randomly sampled. In total, five meat samples of about one kg of each were collected. Two *suusac* samples, each of about one litre, from different containers, were collected randomly from purposively sampled vendors trading along the main street of Eastleigh. The samples were placed in a sterile plastic container and homogenised by gently shaking for about one minute. The meat and *suusac* samples were placed and transported in refrigeration boxes maintained at 4°C. Analysis for LAB and Enterobacteriaceae was conducted immediately on arrival to the laboratory.

### Screening and isolation of Lactic Acid Bacteria

Of each *suusac* sample, 25 mL was inoculated in 225 mL of a physiological saline solution (0.85% NaCl/L (Loba Chemie, India)), homogenised then serially diluted. From the dilutions of $10^{-6}$, $10^{-7}$ and $10^{-8}$, 1 mL of each was pour-plated on an MRS agar (Merck, Germany). The inoculated agar plates were incubated in anaerobic jars with a gas generating kit (Oxoid, England) for three days at 30 °C. One submerged or surface colony that was compact or feathery, small, opaque and white was selected from plates with colony count ranging between 30 and 300 and streaked twice on MRS agar for isolation. In this way, 10 colonies were selected. The pure

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isolates were then tested for morphology, Gram staining and catalase reactions. The isolates that were rod-shaped, gram positive and catalase negative were presumed to be LAB (De Man et al., 1960). The isolates were stored at –18°C for further *in vivo* inhibition trials.

**Screening isolation and biochemical characterisation of Enterobacteriaceae**

Of each meat sample, 25 g was mixed with 225 mL physiological saline solution (0.85% NaCl) and blended. Of the dilutions $10^{-2}$, $10^{-4}$ and $10^{-6}$, 1 mL of each was each spread on VRB agar plates (Oxoid, England) and then incubated at 30 °C for 24 h. Two colonies from plates with colony count ranging between 30 and 300 were picked on the basis of their morphology. In this way, a total of 20 presumptive *Enterobacteriaceae* colonies were selected considering their morphological characteristics as described (Forsythe, 2010). These included seven red (surrounded by reddish precipitation with zones diameter of 1-2 mm), seven pink and six colourless colonies. The isolates were double streaked on VRB agar then incubated at 30 °C for 24 h. Pure colonies were screened for their morphology, Gram staining and oxidase tests as described (Stiles and Ng, 1981a, 1981b). The isolates that were rod-shaped, gram negative and oxidase negative were stored below –4 °C until further biochemical characterisation and *in vivo* inhibition analysis.

For the biochemical characterisation, the stored cultures were thawed and fresh cultured on PCA (Oxoid, England) slants. The slants were incubated at 35 °C for 24 h. The isolates were characterised as described (Barrow and Feltham, 1993). The following tests were carried out: Indole Methyl Red and Voges-Proskauer (IMViC) reaction, utilisation of Simmons citrate (HiMedia, India), decarboxylation of lysine broth (HiMedia, India), growth in KCN broth (HiMedia, India), and deamination of Phenylalanine agar (HiMedia, India). Besides this, few other tests were conducted: fermentation of glucose and lactose, gas production after 24 or 48 h and motility on TSI agar (Oxoid, England), and hydrogen sulphide production on TSI agar (Oxoid, England) and SIM agar (Oxoid, England). The tests were interpreted as positive/negative (+/–).

**Anti-microbial activity of suusac LAB against raw came camel meat *Enterobacteriaceae***

The frozen LAB were resuscitated by thawing followed by transfer into fresh MRS broth, which was then incubated at 30°C for 24 h. The evaluation of the antimicrobial activity against the isolated *Enterobacteriaceae* was carried out using the agar well diffusion assay. Petri dishes containing Mueller-Hinton agar (Sigma, Spain) were dried overnight. The overnight cultures of *Enterobacteriaceae* and LAB grown in BHI broth (Oxoid, England) and MRS broth respectively were standardised to OD (optical density) 0.1 at 600 nm. Aliquots of 100 µL of the standardised

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*Enterobacteriaceae* culture were inoculated and spread on the Mueller-Hinton agar plates. The plates were allowed to dry for 2 h. Four 3 mm holes were made into the agar using a sterile cork borer, and were filled with 25 µL of the standardised culture of the test LAB. The plates were incubated at 30 °C. After 24 h, the diameters of the clear zone around the holes were measured.

**Statistical analysis**

The antimicrobial activity of LAB isolates against *Enterobacteriaceae* isolates was statistically analysed by using analysis of variance (ANOVA). The multiple comparison of statistically significant means was performed using Duncan's separation technique ($P$=0.05). SPSS version 20 (IBM Corp., 2015) was used for the statistical analysis

**RESULTS**

**Screening and isolation of Lactic Acid Bacteria**

From the ten isolates obtained from *suusac*, seven different LAB strains were positively identified. The seven isolates were gram positive and catalase negative rods.

**Screening, isolation and biochemical characterisation of Enterobacteriaceae**

Of the 20 meat isolates purified on VRBA, only 10 were characterised. The characteristics of the ten isolates are given in Table 1. Eight of these ten isolates (EB1, EB2, EB3, EB4, EB7, EB8, EB9 and EB10) fermented glucose and lactose. An acid over acid (A/A) pattern was produced in TSI agar. On the other hand, only EB9 showed IMViC – + – – reaction pattern, whereas the rest showed an IMViC reaction pattern of + + – –. Conversely, only EB1 produced hydrogen sulphide gas in both TSI agar and SIM agar. The other two isolates, EB5 and EB6, fermented glucose but not lactose. They produced an alkaline over acid (Ak/A) reaction pattern in TSI agar. EB5 differed from EB6 by showing positive motility reaction in TSI agar and positive citrate reaction in Simmons' citrate agar. The isolates were further grouped into five comprising of four genera (Figure 1): *Escherichia* spp., *Citrobacter* spp., *Shigella* spp. and *Salmonella* spp. An isolate was considered belonging to a particular genus if it possessed 80% of the characteristics of the genus (Stiles and Ng, 1981a).

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**Table 1: Biochemical characteristics of *Enterobacteriaceae* isolates from camel meat**

| | | | TSIA | | | | $H_2S$ production | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate | Oxidase test | Gram test | Reaction | Gas after 24h | Gas after 48h | Motility | TSIA | SIMA | Indole production | MR | VP | Citrate utilization | LD | PAD | Growth in KCN broth |
| EB1 | – | – | A/A | + | | + | + | + | – | + | – | + | – | – | – |
| EB2 | – | – | A/A | + | | + | – | – | + | + | – | – | – | – | – |
| EB3 | – | – | A/A | – | + | + | – | – | + | + | – | – | + | – | – |
| EB4 | – | – | A/A | + | | + | – | – | + | + | – | – | – | – | – |
| EB5 | – | – | Ak/A | – | + | + | – | – | – | + | – | + | – | – | – |
| EB6 | – | – | Ak/A | - | | – | – | – | – | + | – | – | – | – | – |
| EB7 | – | – | A/A | + | | – | – | – | + | + | – | – | + | – | – |
| EB8 | – | – | A/A | – | + | + | – | – | + | + | – | – | + | – | – |
| EB9 | – | – | A/A | + | | + | – | – | – | + | – | – | + | – | – |
| EB10 | – | – | A/A | + | | + | – | – | + | + | – | – | + | – | – |

TSIA, Triple Sugar Iron agar; SIMA, Sulfide Indole Motility agar; MR, Methyl red test; VP, Voges–Proskauer test; LD: Lysine decarboxylation; Phenylalanine Deamination Ak/A, Alkaline over acid reaction; A/A, Acid over acid reaction;

+ Positive reaction

- Negative reaction

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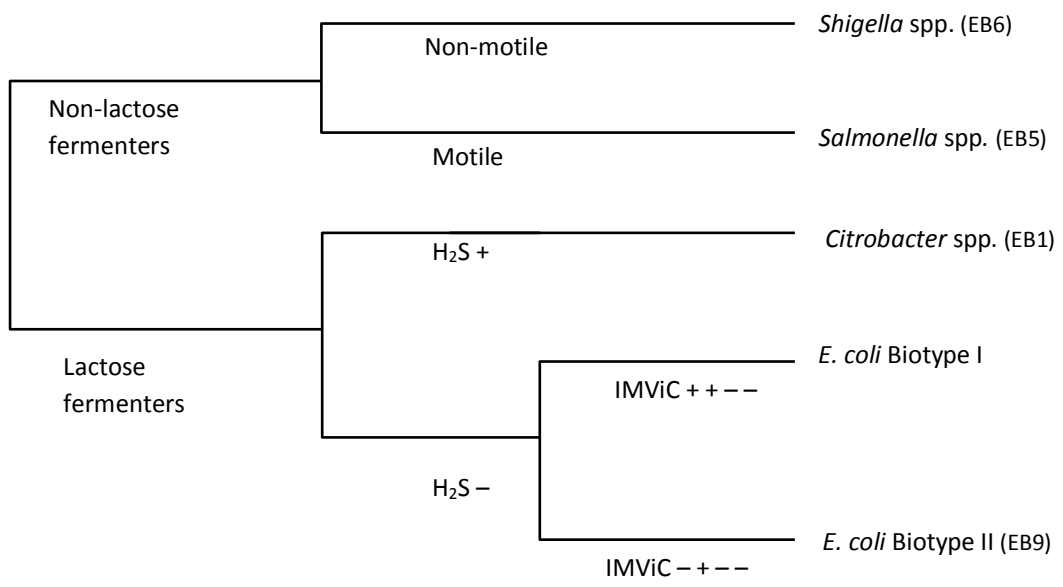


**Figure 1: Genera of camel meat *Enterobacteriaceae* isolates**

**In vitro inhibition**

The antimicrobial activity of *suusac* isolates on presumptive *Enterobacteriaceae* was evaluated by measuring the diameters of the inhibition zones around the diffusion wells. Five out of the ten meat isolates were inhibited by each of the *suusac* isolates (Table 2). The mean diameters of the inhibition zones ranged from 8.5 to 12.5 mm. The inhibition zones of EB1, EB3 and EB6 did not show any significant difference with any of the *suusac* isolates ($P>0.05$). However, a significant difference of mean diameters of the inhibition zones was observed amongst the LAB isolates inhibiting EB8 and EB9 ($P<0.05$). The inhibited *Enterobacteriaceae* included *E. coli* Biotype II, *Citrobacter* spp. *Shigella* spp. and two out of the five *E. coli* Biotype I. On the other hand, *Salmonella* spp. were not inhibited.

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**Table 2: Inhibitory spectra of LAB isolates exhibiting antibacterial activity against *Enterobacteriaceae***

| | Zone of growth inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G1 | | | | | | G2 | G3 | G4 | G5 |
| LAB isolate | EB2 | EB3 | EB4 | EB7 | EB8 | EB10 | EB9 | EB1 | EB5 | EB6 |
| LA1 | - | 11.5±0.5$^{a}$ | - | - | 9.0±1.0$^{a}$ | - | 9.5±0.5$^{ab}$ | 9.0±1.0$^{a}$ | - | 11.5±0.5$^{a}$ |
| LA2 | - | 11.0±1.0$^{a}$ | - | - | 11.0±1.0$^{b}$ | - | 8.5±0.5$^{a}$ | 8.5±0.5$^{a}$ | - | 10.5±0.5$^{a}$ |
| LA3 | - | 11.5±0.5$^{a}$ | - | - | 9.5±0.5$^{ab}$ | - | 10.5±0.5$^{ab}$ | 9.5±0.5$^{a}$ | - | 11.0±1.0$^{a}$ |
| LA4 | - | 11.0±1.0$^{a}$ | - | - | 9.5±0.5$^{ab}$ | - | 9.5±0.5$^{ab}$ | 8.5±0.5$^{a}$ | - | 10.0±1.0$^{a}$ |
| LA5 | - | 11.5±0.5$^{a}$ | - | - | 9.5±0.5$^{ab}$ | - | 9.5±0.5$^{ab}$ | 10.5±0.5$^{a}$ | - | 11.5±0.5$^{a}$ |
| LA6 | - | 11.0±1.0$^{a}$ | - | - | 10.0±0.0$^{ab}$ | - | 11.0±1.0$^{b}$ | 9.5±0.5$^{a}$ | - | 10.5±0.5$^{a}$ |
| LA7 | - | 12.5±0.5$^{a}$ | - | - | 9.5±0.5$^{ab}$ | - | 9.5±0.5$^{ab}$ | 9.5±0.5$^{a}$ | - | 11.5±0.5$^{a}$ |

Mean values with different superscript lower case letters within a column were significantly different ($P<0.05$).
G1, *E. coli* Biotype I; G2, *E. coli* Biotype II; G3, *Citrobacter* spp.; G4, *Salmonella* spp.; G5, *Shigellla* spp.
- No inhibition

## DISCUSSION

There are no studies so far investing the protective effect of microbial cultures adapted to the raw camel meat production environment. In the present study, presumptive LAB were isolated from *suusac* and their potential as protective cultures against presumptive *Enterobacteriaceae* in raw camel meat was demonstrated.

LAB predominates in microflora of *suusac* (Lore et al., 2005). The isolation and characterisation of LAB from other traditionally fermented camel milk products indigenous to other regions, such as Morocco and Sudan, have also been carried out. The predominant isolates in our study were rod-shaped. Similar predominant micro-organisms were isolated from *suusac*, where the predominant species were also *Leuconostoc mesenteroides* subsp. *mesenteroides* (24%) and *Lactobacillus plantarum* (16%) (Lore et al., 2005). The rod-shaped bacteria have also been reported to predominate in other traditional fermented camel milk products (Rahman et al., 2009; Sulieman et al., 2006). The use of MRS agar at 30°C in the isolation and purification of LAB may have resulted in the isolation of mesophilic bacilli LAB while screening against cocci-shaped LAB such as *Lactococcus* spp. MRS agar is a good culture medium for *lactobacilli* (De

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Man et al., 1960). On the other hand, Elliker agar and M17 agar are most suited for the isolation of cocci-shaped LAB (Elliker et al., 1956; Terzaghi and Sandine, 1975).

The four genera of *Enterobacteriaceae* present in raw camel meat pose a serious health risk to consumers. because they significantly to the global cases of foodborne diseases (Al-Mutairi, 2011). Contaminated raw meat is nonetheless one of the main sources of food-borne illnesses (Bhandare et al., 2007; Pidcock et al., 2007). Although, most microbial contaminants of carcasses represent commensal bacteria, some of them such as *Salmonella* spp. and *E. coli* O157:H7 are still a serious threat to consumers (Gustavsson and Borch, 1993; Samelis et al., 2001). Some disease outbreaks were traced to non-O157 Shiga Toxigenic *E. coli* serotypes, especially in Europe, Australia, and Asia (Acheson and Keusch, 1996; Goldwater and Bettelheim, 1995; Rüssmann et al., 1995). In addition, strains belonging to E. *coli* Biotype I were shown to induce lesions characteristic for attaching and effacing E. *coli* in rabbits (Peeters et al., 1988).

*Salmonella* spp. are recognised as one of the most common pathogens causing foodborne gastroenteritis world-wide (Forshell and Wierup, 2006; Wegener et al., 2003). Studies have indicated that *E. coli* and *Salmonella* spp. survive on surfaces for hours or even days after initial contact with the microorganisms (Jiang and Doyle, 1999; Kusumaningrum et al., 2003; Scott and Bloomfield, 1990). The presence of the two genera on camel meat surface can make cross contamination between the meat, meat contact surface and humans more likely.

*Citrobacter* spp. are also commensal organisms of attention given that ingestion of food contaminated by them may cause gastrointestinal infections in healthy persons (Kaclíková et al., 2005). They are also known to produce toxins and contain genes encoding toxins (Guarino et al., 1989; Schmidt et al., 1993). *Shigella* spp. are important pathogens responsible for diarrhoeal diseases and dysentery occurring all over the world (Niyogi, 2005). In addition, the morbidity and mortality due to shigellosis are especially high among children in the developing countries (Niyogi, 2005). This is worsened further as no vaccines against Shigellosis infection are currently available despite the continuously elevating antibiotic resistance of *Shigella* spp. over the years (Khan et al., 2009; Niyogi, 2005).

In this study, we have presented a novel approach to tackle the health risk associated with contaminated camel meat by using LAB isolated from *suusac*. The LAB isolates exhibited a promising growth inhibitory potential against at least half of the *Enterobacteriaceae* isolates of camel meat. Of the six *E. coli* isolates, three were successfully inhibited. In contrast, one study found that all *E. coli* isolates were inhibited by LAB (Kalalou et al., 2004). The production and release of anti-microbial molecules into the extra-cellular milieu is known to vary with factors

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such as substrate composition, cell density and population kinetics (Signorini et al., 2006). This might account for the observed differences between the two studies. The referred study was conducted in a fermented camel meat system while the present one was in an *in vitro* system. Such differences may also lead to the absences of the inhibition of *Salmonella* spp. In accordance with another study (Kalalou et al., 2004), *Citrobacter* spp. were also inhibited by the LAB isolates in this study. *Shigella* spp., which was not investigated in a previous study (Kalalou et al., 2004), was inhibited in the present study.

The inhibitory mechanism of the *suusac* LAB can be attributed to the production of several antibacterial substances such as organic acids (e.g. lactic acid and acetic acid), peroxides and bacteriocins (Kalalou et al., 2004). The inhibitory mechanisms for some of these substances have been described up to certain level (Byczkowski and Gessner, 1988; Einarsson and Lauzon, 1995; Mills et al., 2011; Signorini et al., 2006). LAB originally isolated from meat have been thought to be better candidates for improving the microbiological safety of these foods because they are well adapted to the meat conditions than the LAB isolated other sources (Ammor et al., 2006). However, LAB eventually result into a fermentation process as seen in the case of minced camel meat (Kalalou et al., 2004). This may not be desirable for the fresh meat consumers. Unlike other processed meat products, such as fermented sausages that have other substrates added to them, raw meat is not a suitable substrate for LAB growth. This may explain the few studies involving raw meat systems. To augment this, LAB from already fermented products can successfully be applied as demonstrated herein

The predominance of pathogenic *Enterobacteriaceae* in camel meat demonstrates that pathogenicity can be associated with the meat microflora. It is, therefore, highly desirable to improve meat safety. The present study demonstrated that LAB from *suusac* may repress the growth of some *Enterobacteriaceae* from meat. As protective cultures, LAB may thus be used to influence the microbial ecosystem dynamics and diversity of raw camel meat, consequently improving its safety. Nevertheless, further studies involving diverse LAB from *suusac* and/or their metabolites and different species or even serotypes of the *Enterobacteriaceae* need to be carried out to further characterise the interaction between these two groups, especially in the raw camel meat models.

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