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Antimicrobial Activity of Selected Probiotic Bacteria Isolated from Sudanese Traditional Dairy Product “Rob”

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Abstract: This study was carried out to evaluate the antibacterial activity of selected probiotic strains isolated from the Sudanese traditional fermented dairy food ‘Rob’. Twenty seven samples of Rob were used in this study. The strains were identified by using morphological, physiological and biochemical methods. The isolates of Lactic Acid Bacteria (LAB) were identified as *Lactococcus lactis*, *Lactobacillus delbrecukii* and *Enterococcus faecium*. Cell-free supernatants (CFS) were obtained from the isolates and screened for their antibacterial activity. Pathogenic bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *E.coli* were used as targets. The CFS inhibited the growth of a range of Gram-positive and Gram-negative microorganisms. This result indicated that these probiotic strains could be used as therapeutic agents.

Key words: Lactic acid bacteria, antimicrobial activity, probiotic, Rob

INTRODUCTION

Sudan is one of the biggest wealthy countries that have natural animal and dairy products resources. This represents a source of uncovering strains with probiotic potential, besides presenting promising challenges for dairy industry.

Probiotic bacteria is new field of investigation and a promising area according to its documented healthy beneficial effects. In the food manufacturing processes, Lactic Acid Bacteria (LAB) have a very ancient history of use. Detailed knowledge was obtained from the intense studies of the diverse group of LAB. In the development of probiotic (functional) foods intended for human consumption, strains of LAB such as *Lactobacillus*, *Bifidobacterium* and *Enterococcus* have been most commonly used (Ouweland *et al.*, 2002). This is primarily due to the perception that they are desirable members of the intestinal microflora (Berg, 1998). In addition, these bacteria have traditionally been used in the production of fermented dairy products and generally regarded as ‘safe’ status (O’Sullivan *et al.*, 1992).

The “probiotic concept” remains controversial, especially on mechanisms of actions *in vivo* compared to *in vitro* (Fuller, 2006). Various mechanisms have been proposed to explain the beneficial effects of probiotics, such as antagonism toward pathogens, competition for adhesion sites, struggle for nutrients, enzymatic contribution to digestion, improvement of water quality and stimulation of host immune responses (Tinh *et al.*, 2007).

Probiotics maybe alternative to antibiotics. *Lactobacilli*, *Bifidobacteria*, *Enterococci* and *Streptococci* have been

used prophylactically to prevent traveler’s diarrhea caused by Enterotoxigenic *E. coli* (Cunningham-Rundles *et al.*, 2000) and used as therapeutic agents against diarrheal diseases caused by Rotavirus and *Clostridium difficile* (George and Cummings, 1999).

Antimicrobials have been used increasingly as a primary intervention for inhibition or inactivation of pathogenic microorganisms in foods (Davidson and Zivanovic, 2003). Generally, food antimicrobial agents are not used alone to control foodborne pathogens, but are also tested as substitute to antibiotic.

Rob is made from fermentation of cow, sheep and goat’s milk. It mainly produced in the rural areas. Rob making is one of the traditional way of the Sudanese nomads for the processing of surplus milk (Abdelgadir *et al.*, 2001).

Although many dairy product studies have been conducted in Sudan, few studied the antibacterial activity of the LAB. This article aimed to present new data about the role of the isolated LAB strains to be used as therapeutic agents in Sudan.

MATERIALS AND METHODS

Samples used: Twenty seven samples of traditional cow’s soured milk (Rob) were used in this study.

Source of samples: Samples were randomly collected from five different sites in Khartoum State.

Collection procedure: Rob samples were collected aseptically in sterile containers. The collected samples were transported to the Central Laboratory, Ministry of

Science and Technology, Khartoum, where the analysis was done.

Isolation and identification of Lactic Acid Bacteria:

Samples were cultured on Elikar agar media (HiMedia) and incubated at 37°C for 48 hrs. Then single colonies were subcultured on M17 and MRS (de Man Rogosa and Sharp) agar media (HiMedia), and incubated at 37°C for 48 hrs. The identification was carried out using conventional method such as colony morphology, cultural characteristics, cell morphology, Gram stain reaction, Catalase and biochemical properties as described by Barrows and Feltham (2003). Rapid and standardized API system, API 50 CHL medium and API 50 CH strip, (bioMérieux, Inc. France) were used as confirmatory method for the selected isolates.

Assay for antimicrobial agent: Cell-free supernatants (CFSs) were prepared by growing the isolates in MRS broth at 37°C for 24 h. Then CFSs were obtained by centrifugation at 12000g for 10 minutes at 4°C. *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Salmonella typhi* were used as target pathogens to test the antibacterial sensitivity of the isolates.

The antimicrobial activity of the CFS was determined by the agar well diffusion assay according to the method of Tagg *et al.* (1976). The pathogens were spread over Petri dishes contain Muller- Hinton agar (HiMedia). Wells were made and the supernatants were poured on the wells. The plates were then incubated at 37°C and followed-up for clear zones.

RESULTS AND DISCUSSION

Sudan has one of the largest and most species diverse livestock populations in Africa that contribute greatly to national economy and export revenue. Only little research was carried out on the Sudanese fermented food in general and dairy products in particular (Abdelgadir *et al.*, 2001). Most of the researchers focused on the isolation of the LAB presented in the traditional dairy products. Gap exists in our knowledge regarding mechanisms of action of different Sudanese dairy isolates, and the mechanisms by which dairy foods are fermented.

The use of probiotic bacteria is not documented in the Sudanese literature. Up to this study, there is no publication carried out to study the therapeutic, functional role or even the bacteriocins activity. However, the first Sudanese study to use probiotic bacteria is Kabeir *et al.*, (2004) in a study carried out in Malaysia.

The mechanisms by which probiotics beneficially affect human health are divided into many categories, including strengthening of the intestinal barrier, modulation of the immune response, and antagonism of pathogens either by the production of antimicrobial compounds or through competition for mucosal binding

Against *Staphylococcus aureus*

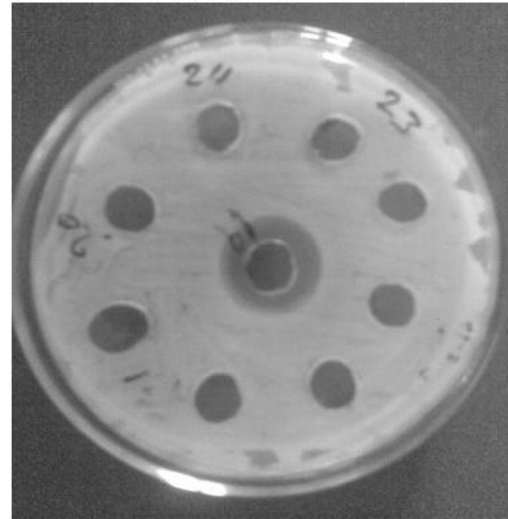


Fig. 1: Inhibition zones of potential gastrointestinal pathogens by cell free supernatants. 19. *Lactobacillus delbrecukii*

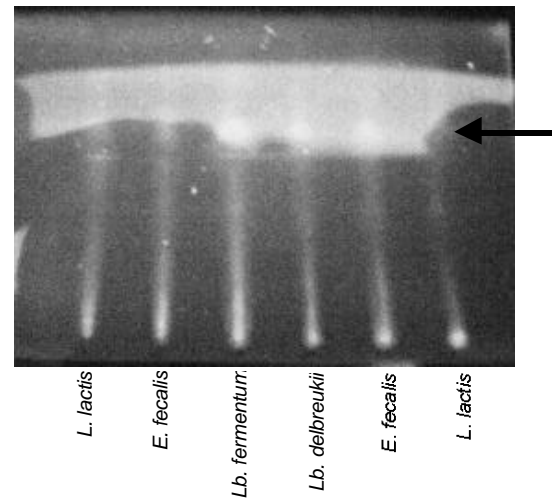


Fig. 2: TLC of Bacteriocin. Arrow shows the separation peak using the UV light

sites (Saxelin *et al.*, 2005; Mercenier *et al.*, 2003). Although there is suggestive evidence for each of these functional claims, the molecular details behind these mechanisms remain almost entirely unknown (Marco *et al.*, 2006).

In this study three strains *Lactococcus lactis*, *Enterococcus faecium*, *Lactobacillus delbrecukii* showed great inhibition zones (Fig. 1) to the pathogenic microbes, notably, *E.coli*, *Staph.aureus* and *Klebsiella pneumoniae*. As universally used in the publications, the presence of inhibition zone assumed a good therapeutic indication of the strain. But to reach this end, many chemical tests are needed such as detection by HPLC

and GM-LC. The CFSs were detected using Thin Layer Chromatography (TLC) technique (Fig. 2). TLC is a simple, quick, and inexpensive procedure that gives a quick answer to how many components are in a mixture. To test the purity of the CFSs, the following mobile phases were tested; chloroform, methanol and methanol 60% and chloroform 40%. The solvent was 18cm and the peak (*R_f*) of the bands of all of the isolates was 10.5. Bacteriocins are antimicrobial proteinaceous compounds that inhibit the growth of both Gram-positive and Gram-negative bacteria (Yildirim and Johnson, 1997). Nisin is the most extensively characterized bacteriocin of antimicrobial proteins produced by LAB (Jack *et al.*, 1994). Nisin is rapidly detected by gene amplification. But PCR cannot amplify a new gene. The technique used in this study did not make comparison between bacteriocin (extracted from the strains) and known antibiotics.

Conclusion: Further research on identification and molecular characterization of the isolates from milk and dairy products are needed. The coming researches of Sudanese traditional foods may uncover a number of probiotic and/or prebiotics to give highly healthy beneficial effects. The revealing of new strains with probiotic potential will present interesting challenges for dairy industry, as not all organisms survive in milk products, or produce a suitable, shelf-stable and flavourful product. The next generation of dairy products may contains a variety of bacteria (probiotic) or its products (prebiotics) in order to give a potential healthy, immunological and therapeutic benefits.

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