

Antimicrobial activity of Some Lactic Acid Bacteria strains isolated from Sudanese fermented foods

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The present study screened thirty lactic acid bacteria isolates from ten samples of Sudanese traditional fermented foods collected from different locations in Khartoum state for antibacterial activity. Microscopic examination revealed that all the isolates are Gram positive, twenty nine (96.3%) of these isolates were rod-shaped, and the rest (3.3%) is cocci. All isolates were subjected to a number of preliminary tests, which are commonly used for the identification of lactic acid bacteria these including morphological, physiological and biochemical. The results indicated all isolates are LAB, and most of the tested 30 isolates produced exopolysaccharides from sucrose under the conditions used. These isolates can be utilized for the production of safe fermented foods and milk products. Antimicrobial activities of the 30 isolates were tested against five pathogenic bacterial strains using the agar disk diffusion technique. Out of these 25 (83.3 %) isolates exhibited antimicrobial activity against at least one indicator strain tested, six isolates were characterized to be producers of antibacterial as evidenced by strong inhibition zones formed against sensitive organisms on agar plates, the isolates demonstrated abroad spectrum of microbial activity by inhibiting *Staphylococcus aureus* ATCC 43306, *E. coli* ATCC 25922 and *Salmonella typhae*. Five isolates (16.7 %) had no antimicrobial activity. The physiological results, biochemical tests and sugar fermentation profiles all gave the same results for the bioactive isolates, which were indicative of bacilli and enterococci.

Key words: Antimicrobial compounds, lactic acid bacteria, fermented foods.

INTRODUCTION

Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic acid fermentations (Oyetayo *et al.*, 2003). Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics (Leroy, 2004). Various types of lactic acid bacteria produced bacteriocins have been identified and characterized (Hernandez *et al.*, 2005; Badarinath and Halami, 2011). One important reason for screening probiotic lactic acid bacteria is the ability of some strains to produce antimicrobial compounds, including organic acids, hydrogen peroxide and bacteriocins, that can inhibit the growth of enteric pathogens. Bacteriocins are antimicrobial proteinaceous substances that are produced by some bacteria against other bacteria that are closely related to the producing organisms growing in the same medium (Ruiz-Larrea *et al.*, 2007). A number of studies have reported the antagonistic properties of probiotics against many

common gastroenteric pathogens, e.g. *Salmonella sp.*, *Escherichia coli* O157:H7 (Brashears *et al.*, 2003), *Clostridium perfringens* (Kim *et al.*, 2007), *Campylobacter jejuni* (Chaveerach *et al.*, 2004), *Listeria monocytogenes* and *Helicobacter pylori* (Mukai *et al.*, 2002). The bacteriocins from the GRAS lactic acid bacteria have risen a great deal of attention to control pathogens in foods. Lactic acid bacteria exert strong antagonistic activities against many microorganisms, including food spoilage organisms and pathogens. The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food-borne pathogenic microorganisms (Schillinger *et al.*, 1996). For the last decades, the research focus has been on the naturally occurring antimicrobial, like bacteriocins as an additional hurdle to fight the unwanted bacterial growth and prevent food spoilage (Du Toit *et al.*, 2002; Bauer *et al.*, 2003). The aim of this study is to isolate, characterize and screen lactic acid bacteria strain for

producing antibacterial activity against pathogenic bacteria from some Sudanese fermented foods.

MATERIALS AND METHODS

Sampling

Ten samples of traditional Sudanese fermented foods including fermented milk, fermented vegetables were collected from different locations in the Khartoum, and the samples were collected in 250-ml sterile screw-cap bottles kept in a refrigerator (4 °C) until the time of sampling.

Isolation of Lactic Acid Bacteria and Culture Conditions

From each sample, serial dilutions were prepared and 100µl from the 10⁻³ - 10⁻⁷ dilution of the samples were inoculated on plated of MRS Agar medium (de Man, Rogosa and Sharp 1960). The medium consisted of: Casein peptone tryptic digest 10g, meat extract 10g, yeast extract 5.0g, glucose 20.0g, K₂HPO₄ 2.0g, sodium acetate 5.0g, di-ammonium citrate 2.0g, MgSO₄·7H₂O 0.2g, MnSO₄·H₂O 0.05g., and distilled water 1000 ml. The pH was adjusted to 6.2 - 6.5 according to Hitchner *et al.*, (1982). All plates were incubated at 37°C for 2-3 days under anaerobic conditions using anaerobic jars with a gas generating kit (Oxoid, BR 38).

Purification and Preservation of LAB Isolates

Bacterial colonies from the plates of the appropriate dilution were picked at random for characterization, based on differences in morphology (size, form and shape). The different isolates were tested for their Gram reaction, catalase and oxidase activity. Only Gram- positive, catalase and oxidase negative isolates were purified by Streak plate method (Srinivasan *et al.*, 2008). Onto the same agar medium (MRS) before being subjected to preliminary identification. Pure and active LAB isolates were stored in the short term (up to 3 months) at 4°C on agar slopes.

BIOASSAYS

Target Microorganisms

Several pathogenic microorganisms were used to screen the antimicrobial activity of the LAB isolates. Bacterial target strains were grown over night at 37°C in nutrient agar. The test-microorganisms used for screening of antimicrobial activity were Gram-positive bacteria which included *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8263), and the Gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella typhie* (IMD 39).

Screening for Antimicrobial Activity of Lactic Acid Bacteria Isolates

An overnight culture of each isolate grown in MRS broth at 37 °C was standardized to an optical density of 0.5 at a wavelength of 600 nm. (Hitachi U-2010 model spectrophotometer). One percent of standardized culture was used to inoculate MRS broth. After incubation at 37 °C for 24 hours, the stationary phase grown culture were centrifuged at 14,000 rpm for 10 minutes at 4°C and the resulting extract was adjusted to pH 7.0 and used to evaluate

antimicrobial activity. An agar well diffusion method as described by (Felming *et al.*, 1975; Pulusani *et al.*, 1979) was used with some modifications. A lawn of an indicator strain was made by spreading the cell suspension over the surface of TSA plates with a cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 10.0 mm was used to cut uniform wells in the agar plates. Each well was filled with 100 µl of filter-sterilized supernatant obtained from culture grown in MRSC medium. All the assays were carried out in triplicate. After incubation at 37°C for 24 hrs, the diameter (mm) of the inhibition zone around the well was measured. Resistance was defined as the absence of a growth inhibition zone around the discs.

Identification of the bioactive lactic acid bacteria

Physiological and biochemical characteristics were used to identify the active LAB isolates, for the physiological tests, LAB isolates were evaluated for the production of gas (CO₂) from glucose, growth in different concentrations of NaCl using the method of Andrighetto *et al.*, (2001). Biochemical characteristics were established by performing the enzymatic tests and the sugar fermentation test using API 50 CHL test kit (bioMerieux SA, France). The API test strips were prepared as recommended by the kit supplier and scored after incubation for 24 and 48 hours at 37°C. The results were communicated to the APIWEB, which used the phenotypic data to predict a species identity for each isolate. Interpretations of the fermentation profiles were facilitated by systematically comparing all results obtained from the isolates studied with information from the computer-aided database, in which the identification of a microorganism is accompanied by the following information: (i) The percentage of identification (%ID) is an estimate of how closely the profile corresponds to the taxon relative to all the other taxa in the database. (ii) The T-index represents an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon. Its value varies between 0 and 1, and is inversely proportional to the number of atypical tests. (iii) Comments on the quality of identification derived from the %ID and the T-index of the selected taxon (excellent identification %ID > 99.9 and T > 0.75).

Shape of Isolates	No. of isolates	of Gas from glucose	Percentage of total
Rods	14	-	46.7
Rods	15	+	50
Cocci	1	-	3.3
Total	30		100

Table 1: Major groups of the 30 LAB isolates obtained from some Sudanese fermented foods samples

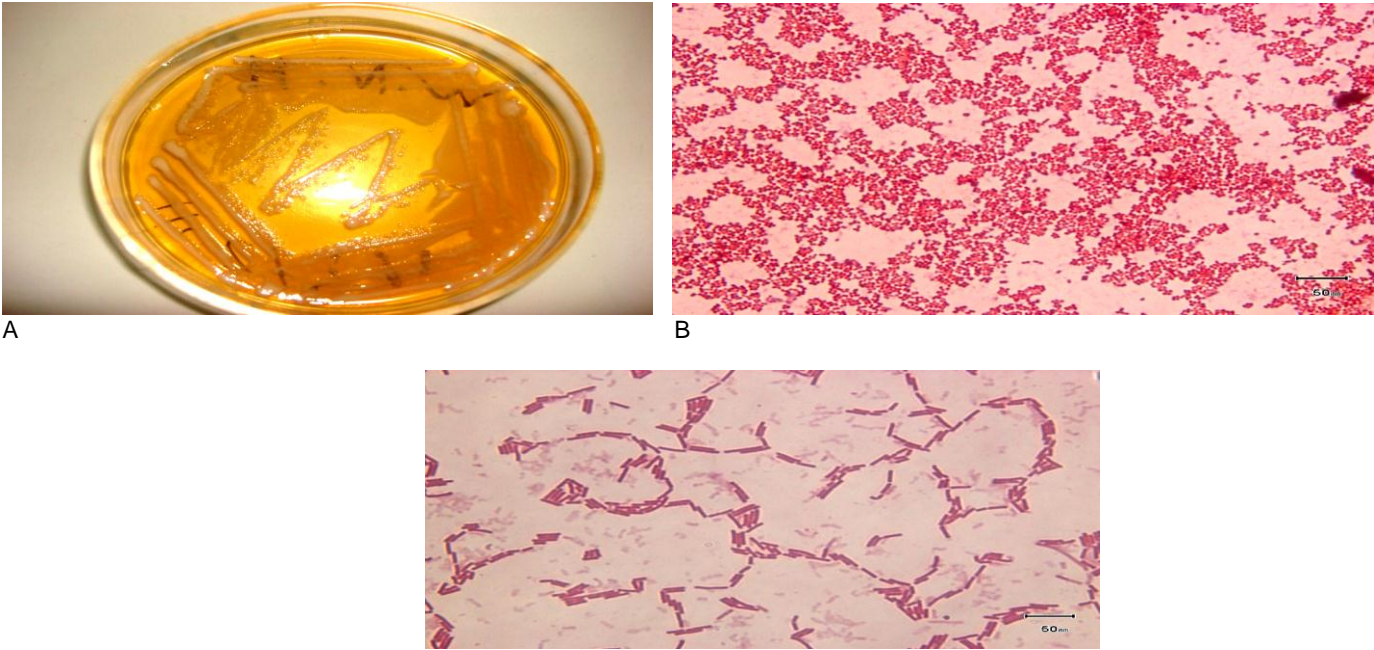


Figure 1: A: Gram staining of Enterococcus sp. Showing cocci shaped, B: Gram staining of Lactobacillus spp. showing rod shaped, C: colony morphology of LAB

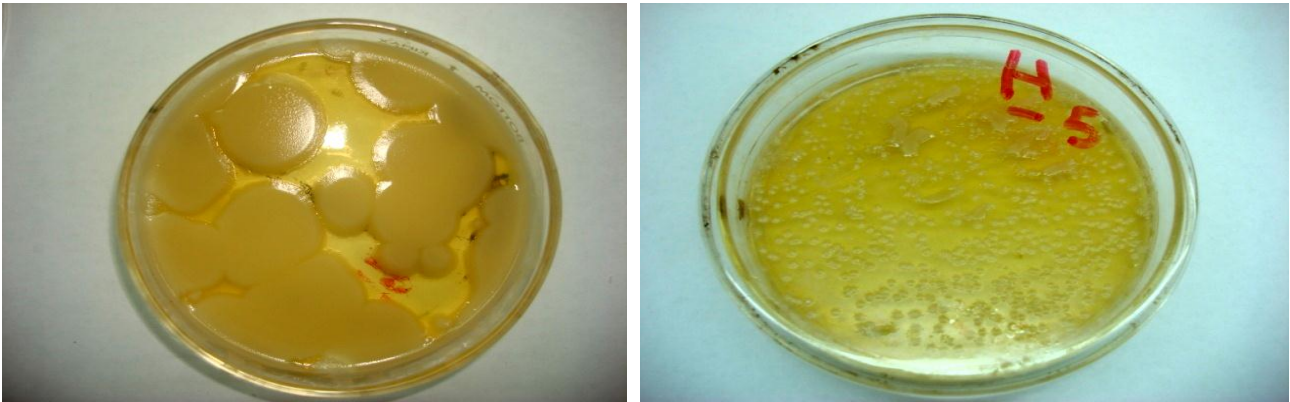


Figure 2: Production of EPS by LAB isolate A- showing EPS production B. control

Lactic acid bacteria isolates																														
Test organisms	Inhibition zone in mm																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Staphylococcus aureus</i> ATCC 43306	22	14	23	20	00	18	18	17	20	21	25	00	18	20	19	16	20	00	00	00	00	00	00	00	00	00	00	00	00	00
<i>Escherichia coli</i> ATCC 25922	20	00	00	00	00	00	20	24	22	00	25	00	00	25	15	18	15	00	00	20	00	00	23	00	00	18	00	00	00	20
<i>Salmonella typhae</i> (IMD 39).	25	00	20	00	00	22	00	00	00	18	30	00	25	31	20	32	27	20	18	20	16	00	21	00	17	18	00	18	18	20
<i>Pseudomonas aeruginosa</i> (ATCC 27853),	32	00	00	12	00	16	00	11	19	00	25	00	00	42	25	22	14	12	00	12	00	00	15	00	00	12	17	00	00	00
<i>Bacillus subtilius</i> (NCTC 8263),	21	14	00	00	00	00	13	14	00	00	28	00	12	27	23	21	25	00	00	00	14	00	00	00	14	00	13	00	13	14

Table 2: Antimicrobial activity of the isolated LAB using culture supernatant against different target organisms

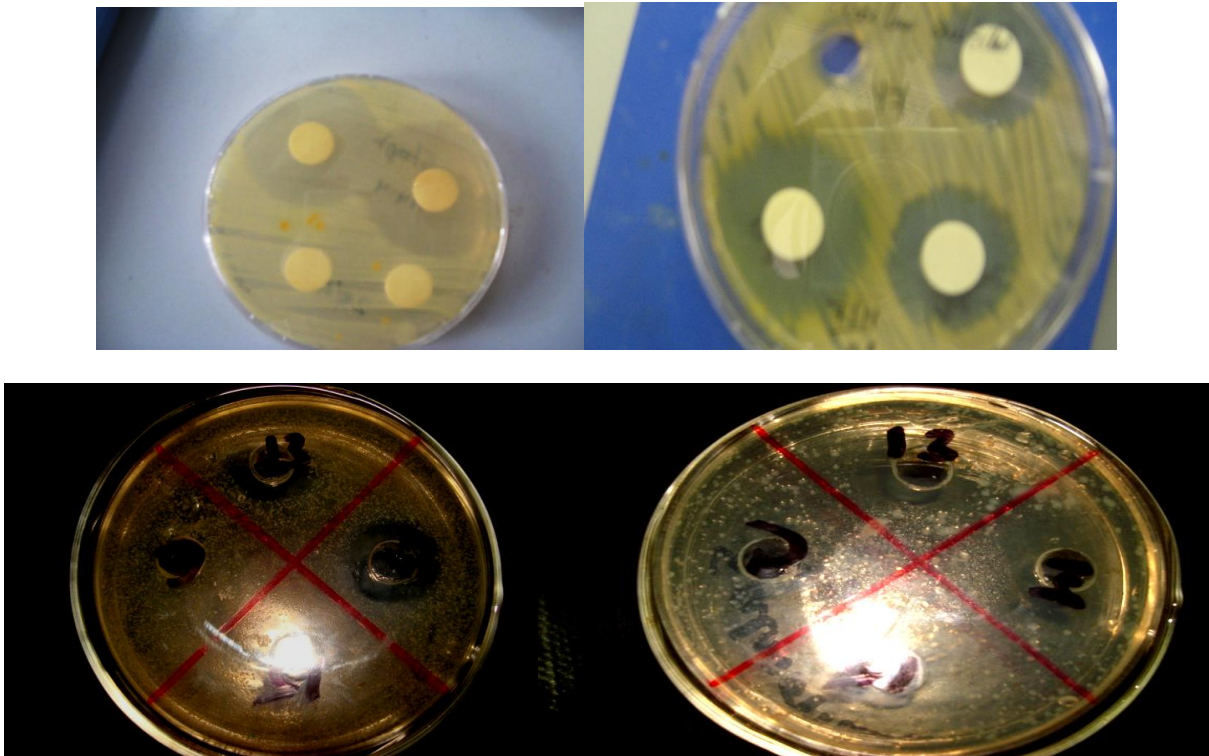


Figure 3: Agar disk and agar well bioassay of lactic acid bacteria isolates showing the antibacterial activity against pathogenic bacteria

RESULTS AND DISCUSSION

Isolation and characterization of lactic acid bacteria

Lactic acid bacteria were isolated from 10 samples of some Sudanese fermented foods using modified MRS supplemented with 0.05% L-cysteine-hydrochloride (MRSC). L-cysteine-hydrochloride was used as a reducing agent to provide more strict anaerobic conditions to MRS medium which will promote the growth of lactic acid bacteria. Lactic acid bacteria could be isolated from all samples and three to four distinct colony morphologies were selected from each sample and screened for Gram-positive and catalase negative bacteria. A total of 30 lactic acid bacteria were finally selected for further analysis. Microscopic examination of the common colony types showed different cell morphologies, cocci, long-rods in chain, rods and short rods. The physiological results, biochemical tests and sugar fermentation profiles gave the same results for the bioactive six isolates. In all samples, short rod-shaped bacteria were found to be the predominant type and the strain was later identified as *Lactobacillus lactis* sp. *Lactis*, *Lactobacillus fermentum*, and *Lactobacillus rhamnosus*.

Screening for antimicrobial activity of lactic acid bacteria isolates

An agar disk diffusion method was used to assess the production of antimicrobial compounds by the selected lactic acid bacteria isolated from some Sudanese fermented foods against three pathogens including Gram-positive, *Staphylococcus aureus* ATCC 43306 and Gram-negative *E. coli* ATCC 25922 and *Salmonella typhae*. Out of 30 isolates tested, 17 isolates were found to exhibit antimicrobial activity against indicator strains. As shown in Table 2, the spectra of inhibition were different among the isolates tested. Isolates 1, 11, 14, 15, 16, and 17 showed the largest antimicrobial spectrum, exhibiting inhibitory activity against all 3 pathogens. Only 5 isolates out of 30 isolates including isolates 5, 12, 22, 24 and 27 have no antimicrobial activity against pathogens tested. To rule out the possibility that the inhibition was due to the effect of organic acids, the supernatants were neutralized to pH 7.0 before the assay. Therefore, antimicrobial activities of these bioactive isolates might be due to the production of bacteriocin-like compounds. These bacteriocin-like compounds might be useful as biological control agents, an alternative to chemical preservatives in food industry. Chemical preservations are used to prevent the growth of food spoilage microorganisms, customer preference for more natural products, and the strict legislation regarding preservatives (Enrique et al., 2007; Ruiz-Larrea 2010). Additional or novel antimicrobial agents are therefore required in foods. All these considerations have increased the interest in research to look for new preservation strategies. Lactic acid bacteria produced bacteriocins play a very important role in the food fermentation industry as natural preservatives, since they are capable of inhibiting the growth of many food spoilage and pathogenic bacteria.

CONCLUSIONS

- In this research, lactic acid bacteria were isolated from Sudanese fermented foods and was found to be the predominant species.

- Carbohydrate fermentation pattern analysis using API50 CHL kit used to identify some species of lactic acid bacteria isolated from Sudanese fermented foods.
- Diversity of lactic acid bacteria with antimicrobial activity from Sudanese fermented foods was assessed by agar well diffusion method.
- Antimicrobial compounds produced by LAB have provided these organisms with a competitive advantage over other microorganisms.
- In conclusions, 25 LAB isolates from the Sudanese fermented foods, capable of producing antimicrobial activity.

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