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# Isolation, Identification and Antimicrobial Activity of Some Local Isolates of Lactic Acid Bacteria

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## ABSTRACT

Seventy one bacterial isolates were obtained from the normal local habitats of Lactic Acid Bacteria (LAB) like babies buccal cavity, woman milk (two weeks after birth), animal milk (cow, goat and camel) and fermented food (pickles). Eighteen bacterial species were isolated from buccal cavity, whereas 53 species from milk and pickles samples. They were characterized and identified through physiological, biochemical tests and API 50CH kit. The isolates belonged to the genera *Lactobacillus* (14 strains), *Pediococcus* and *Lactococcus* (8 strains, each), *Streptococcus* (6 strains) and *Leuconostoc* (4 strains). Genus *Lactobacillus* includes *L. plantarum*, *L. helveticus*, *L. curvatus* and *L. lactis* while *Pediococcus* includes *P. pentosaceus* and *P. acidilactici*. Genus *Lactococcus* was presented by *L. lactis* whereas genus *Streptococcus* was presented by *S. mitis*, *S. sobrinus*, *S. salivarius* and *S. ratius*. Genus *Leuconostoc* includes *L. mesenteroides* and *L. lactis*. Screening of all LAB isolates (71) for antimicrobial activity revealed that 40 bacterial isolates showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The highest activity was achieved by *Streptococcus salivarius*, *Lactococcus lactis*, *Pediococcus pentosaceus* and *Lactobacillus plantarum*. No activity was observed against methicillin resistant *Staphylococcus aureus* (MRSA), *Aspergillus niger and Fusarium oxysporum*.

**Key words:** Lactic acid bacteria, API technique, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*

## INTRODUCTION

A few decades after the introduction of antibiotics into clinical practice, resistance of pathogenic bacteria to them has become a major health concern. Actually, many gram positive and gram negative opportunistic pathogens are becoming resistant to most clinically available drugs (Greenberg, 2003).

New therapeutic drugs are needed to improve the management of microbial diseases (Taylor *et al*., 2002) and consequently there is a renewed interest in discovering novel classes of antibiotics that have different mechanisms of action (Weigel *et al*., 2003). Lactic acid bacteria possess the ability to produce antibiotics which can affect Gram-positive pathogenic bacteria and fungi (Bunch and Harris, 1986; Aktypis *et al*., 2007; Mezaini *et al*., 2009) and some Gram-negative bacterial species (Cardi, 2002).

The multiple nutritional requirements restrict the LAB habitats into nutrients rich ones, such as various food products like milk e.g., goats, cows and camel's milk (Tatsadjieu *et al*., 2009; Ogunshe *et al*., 2007; Mourad and Meriem, 2008) meat (Al-Allaf *et al*., 2009), beverages and vegetables (Ogunshe *et al*., 2007) and olives (Mourad and Nour-Eddine, 2006). LAB also represent the normal flora of infant mouth (Davis, 1955), women's milk (Beasley, 2004), intestines and vagina of mammals (Martin *et al*., 2003; Adolfsson *et al*., 2004).

Accordingly, the present study aimed at isolation of local lactic acid bacteria from their natural habitats. Screening for the antimicrobial activity of the isolated LAB species against some pathogenic bacteria and fungi was investigated. Identification of the active isolates using API 50 CH kits was also adopted.

## MATERIALS AND METHODS

### Isolation of Lactic Acid Bacteria (LAB)

**Collection of samples:** Samples were collected from the normal habitats of lactic acid bacteria like women and animal milk and also from fermented food like pickles. Human samples include woman milk (after two weeks birth) and swabs from babies' buccal cavity (two weeks to 18 months age). Milk samples, were kept in sterile screw capped plastic bottles. Samples were transported in cooler boxes to the laboratory.

**Isolation of bacteria:** Swabs, taken from buccal cavity, were spread directly and streaked over agar surface of sterile Mann, Rogosa and Sharpe (MRS) medium. The plates were incubated at 37°C for 24 h. Concerning milk samples, one milliliter was aseptically transferred to 9 mL sterile dist. water and shaked well to get a dilution of $10^{-1}$. Several dilutions were then made to obtain a proper dilution ($10^{-3}$) with pickle samples, 10 g were placed aseptically in sterile 90 mL dist. water to obtain a dilution of $10^{-2}$ then shaked well. Aliquot of 0.1 mL of each dilution was streaked over plates containing sterile MRS medium. Two plates were performed for each of the isolation samples. After incubation for 24 h at 37°C, the produced bacterial colonies were counted, then purified and preserved at 4°C.

**Identification of isolated lactic acid bacteria to genus level:** The isolated LAB species were identified to genus level by morphological and physiological tests (Gram stain, spore staining, motility, haemolysis oxidase and catalase tests, starch hydrolysis and acidifying activity the change in pH i.e., ApH) according to Bergey's Manual of systematic bacteriology (Holt *et al*., 1994; Sharpe, 1979).

**Identification of bacteria up to the species level using API 50 CH kits:** API 50CH Kits (Biomerieux, Marcy-l'Etoile, France) were used for such purpose. Bacterial isolates were inoculated according to instructions provided by the manufacturer. The APIs were incubated at 37°C and reaction was observed after 24 and 48 h. API database (Biomerieux SA) and accompanying computer software were used to interpret the results.

**Test pathogenic microbes:** Standard local pure cultures of pathogenic *Escherichia coli, Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* were kindly provided by Ohud hospital microbiology lab. *Fusarium oxysporum* and *Aspergillus niger* were provided by Biology department, Faculty of Science, Taibah University and confirmed

by identification according to De Hoog *et al.* (2000) and Toussoun and Nelson (1976) for *Fusarium* species. These cultures were checked up again in terms purity and species characteristics for confirmation.

**Screening of the bacterial isolates for antimicrobial activity:** Antimicrobial activity of the bacterial isolates against test pathogenic strains was determined by well diffusion method on nutrient agar and Sabouraud Dextrose Agar (Haley and Callaway, 1978) for bacteria and *Candida albicans*, respectively while Czapek Dox Agar medium (Smith, 1960) was used for *Fusarium oxysporum* and *Aspergillus niger*. About 20 mL of the sterilized medium was poured into sterile Petri-dishes (9 cm diameter) and allowed to solidify. Aliquot of 0.1 mL of test pathogenic bacterial suspension ($3\times10^8$ cfu $mL^{-1}$) was spread properly onto the agar surface and kept in a refrigerator for 2 h. Wells (7 mm) were cut into the plates and 100 μL of Cell-Free Filtrate (CFF) of the isolated LAB strain (obtained by centrifugation at 8000 rpm for 10 min at 4°C) was placed into each well. Plates were left for diffusion then incubated at 37°C for 24 h in case of bacteria and *C. albicans* (Joshi *et al.*, 2006). In case of *F. oxysporum* and *A. niger* a spore suspension (about $10^6$ $mL^{-1}$) is made up from fungal discs (5 days old). One milhliter of each spore suspension was placed and distributed over Czapek Dox Agar plates and left for 2 h. Wells were made and 0.1 mL of bacterial CFF was placed in the well and plates were incubated at 28°C for 48 h. (Bunch and Harris, 1986). The diameters of the resulting inhibition zones (mm) were measured and the means were calculated and taken as a criterion for the antimicrobial activity.

## RESULTS AND DISCUSSION

**Isolation of Lactic Acid Bacteria (LAB):** In the present study, seventy one bacterial isolates were obtained from the normal habitats of LAB. In case of samples from buccal cavity 18 bacterial species were isolated whereas 53 species were isolated from women and animal milk and fermented pickles. Six, 19, 6, 10 and 12 isolates were recovered from milk of women, cows, goats, camels and fermented pickles, respectively (Table 1). These isolates were given the symbol B ($B_1$, $B_2$, $B_3$,... ... and $B_{18}$) for bacterial isolates from buccal cavity, the symbol W ($W_1$, $W_2$, $W_3$... ..and $W_6$) for bacterial isolates from women milk, the symbol C ($C_1$, $C_2$,$C_3$... ..and $C_{19}$) for bacterial isolates from Cow milk, the symbol G ($G_1$, $G_2$, $G_3$... ..and $G_6$) from Goat milk, the symbol K ($K_1$, $K_2$, $K_2$ ... .and $K_{10}$) from Camel milk and the symbol F ($F_1$, $F_2$, $F_3$... ..and $F_{12}$) from Fermented food pickles. Description of culture morphological and microscopical characteristics was also included in Table 1.

In this connection, natural habitats have always been the most powerful means for isolation of useful cultures applied for scientific, biotechnological and commercial purposes. This is certainly true for LAB which play an important role in a large number of various traditional industries.

Different LAB species were isolated from different habitats like animal raw milk and dairy products El-Soda *et al.* (2003), fermented cow's and goat's milk (Savadogo *et al.*, 2004), processed and fermented food (Nowroozi *et al.*, 2004; Ammor *et al.*, 2006; Joshi *et al.*, 2006), buccal cavity of infant (Davis, 1955) and even infant feces (Li *et al.*, 2008).

**Physiological and biochemical tests of isolated LAB:** All pure bacterial isolates of LAB obtained on MRS medium and isolated from the normal habitats of LAB were Gram+ve, non spore former, non motile, oxidase -ve, catalase -ve except 8 species ($B_1$, $B_4$, $B_{10}$, $B_{14}$, $B_{17}$, $W_3$, $C_9$ and $K_5$) which were weakly catalase+ve (Table 2). Concerning starch hydrolysis all the isolated species were found to hydrolyze starch. With regard to hemolysis test, all the species have no ability to degrade blood but species $B_2$, $B_8$ and $B_{14}$ were α-hemolytic whereas $B_{15}$ and $G_1$ were β-hemolysis.

Table 1: Culture morphological and microscopical characteristics of LAB isolates recovered from buccal cavity, women milk, cow milk, goat milk, camel milk and fermented pickles

| Isolate symbol | Samples | Colony shape | Colony size (mm) | Colony color |
|---|---|---|---|---|
| $B_1$ | Buccal cavity | Round, smooth, convex | 2.0-3.0 | White |
| $B_2$ | | Round, smooth, drop-like | 1.0-2.0 | White |
| $B_3$ | | Round, smooth, convex | 2.0 | White |
| $B_4$ | | Round, smooth, drop-like | 1.0 | White |
| $B_5$ | | Round, smooth, convex | 1.0-2.0 | White |
| $B_6$ | | Round, smooth, raised | 2.0-3.0 | White |
| $B_7$ | | Round, smooth, convex | 1.0- 2.0 | White |
| $B_8$ | | Round, smooth, convex | 2.0- 3.0 | White |
| $B_9$ | | Round, smooth, flat | 2.0-3.0 | White |
| $B_{10}$ | | Round, smooth, convex | 3.0 | White |
| $B_{11}$ | | Round, smooth, raised | 2.0- 3.0 | White |
| $B_{12}$ | | Round, smooth, flat | 1.0-2.0 | White |
| $B_{13}$ | | Round, smooth, drop-like | 2.0 | White |
| $B_{14}$ | | Round, smooth, flat | 1.0-2.0 | White |
| $B_{15}$ | | Round, smooth, convex | 2.0 | White |
| $B_{16}$ | | Round, smooth, flat | 3.0 | White |
| $B_{17}$ | | Round, smooth, drop-like | 3.0 | White |
| $B_{18}$ | | Round, smooth, drop-like | 4.0 | White |
| $W_1$ | Women milk | Round, smooth, flat | 2.0-3.0 | White |
| $W_2$ | | Round, smooth, convex | 2.0-3.0 | White |
| $W_3$ | | Round, smooth, flat | 2.0- 3.0 | White |
| $W_4$ | | Round, smooth, flat | 1.0- 2.0 | White |
| $W_5$ | | Round, smooth, convex | 2.0- 3.0 | White |
| $W_6$ | | Round, smooth, flat | 1.0- 2.0 | White |
| $C_1$ | Cow milk | Round, smooth, raised | 2.0- 3.0 | White |
| $C_2$ | | Round, smooth, convex | 3.0 | White |
| $C_3$ | | Round, smooth, convex | 2.0 | White |
| $C_4$ | | Round, smooth, flat | 3.0 | White-brilliant |
| $C_5$ | | Round, smooth, convex | 3.0 | White |
| $C_6$ | | Round, smooth, flat | 2.0 | Yellowish white brilliant |
| $C_7$ | | Round, smooth convex | 2.0 | yellowish white |
| $C_8$ | | Round, smooth convex | 4.0 | White- brilliant |
| $C_9$ | | Round, smooth, flat | 2.0- 3.0 | White-brilliant |
| $C_{10}$ | | Round, smooth, raised | 2.0- 3.0 | White |
| $C_{11}$ | | Round, smooth, raised | 1.0 | White |
| $C_{12}$ | | Round, smooth, flat | 2.0 | White-brilliant |
| $C_{13}$ | | Round, smooth, convex | 2.0-3.0 | White |
| $C_{14}$ | | Round, smooth, flat | 2.0 | White-brilliant |
| $C_{15}$ | | Round, smooth, convex | 1.0 | White-brilliant |
| $C_{16}$ | | Round, smooth, convex | 2.0 | White |
| $C_{17}$ | | Round, smooth, flat | 1.0 | White-brilliant |
| $C_{18}$ | | Wrinkled, way, hilly | >4.0 | Yellowish white |
| $C_{19}$ | | Wrinkled, irregular | 3.0-4.0 | White-orange |
| $G_1$ | Goat milk | Round, smooth, flat | 2.0-3.0 | White |
| $G_2$ | | Round, smooth, raised | 1.0-2.0 | White |
| $G_3$ | | Round, smooth, convex | 2.0- 3.0 | White-brilliant |
| $G_4$ | | Round, smooth, convex | 1.0-2.0 | White |
| $G_5$ | | Round, smooth, convex | 3.0 | Yellowish white |

Table 1: Continued

| Isolate symbol | Samples | Colony shape | Colony size (mm) | Colony color |
|---|---|---|---|---|
| $G_6$ | | Round, smooth, flat | 2.0-3.0 | White |
| $K_1$ | Camel milk | Round, smooth, flat | 1.0-2.0 | White |
| $K_2$ | | Round, smooth, flat | 2.0-3.0 | Yellowish White |
| $K_3$ | | Round, smooth, convex | 2.0 | White |
| $K_4$ | | Round, smooth, flat | 2.0 | White- brilliant |
| $K_5$ | | Round, smooth, flat | 2.0-3.0 | Yellowish white |
| $K_6$ | | Round, smooth, convex | 2.0-3.0 | White |
| $K_7$ | | Wrinkled, way, hilly | >4.0 | Yellowish white |
| $K_8$ | | Wrinkled, irregular | 3.0-4.0 | White |
| $K_9$ | | Round, smooth, convex | 2.0-3.0 | White |
| $K_{10}$ | | Round, smooth, flat | 2.0 | White |
| $F_1$ | Fermented food | Round, smooth, drop-like | 3.0 | White |
| $F_2$ | | Round, smooth, drop-like | 4.0 | White |
| $F_3$ | | Round, smooth, convex | 3.0 | White |
| $F_4$ | | Round, smooth, drop-like | 2.0 | Yellowish white |
| $F_5$ | | Round, smooth, drop-like | 3.0 | White |
| $F_6$ | | Round, smooth, drop-like | 4.0 | White |
| $F_7$ | | Irregular and spreading | ≈3.0 | Yellowish white |
| $F_8$ | | Round, smooth, convex | 2.0-3.0 | White-brilliant |
| $F_9$ | | Round, smooth, flat | 2.0-3.0 | Yellowish white |
| $F_{10}$ | | Round, smooth, drop-like | 4.0 | White |
| $F_{11}$ | | Round, smooth, flat | 2.0 | White-brilliant |
| $F_{12}$ | | Round, smooth, convex | 2.0-3.0 | White |

Table 2: Primary physiological and biochemical tests of the LAB isolates

| Isolate code | Gram stain | Spore forming | Motility test | Oxidase | Catalase | Hemolysis | Starch hydrolysis |
|---|---|---|---|---|---|---|---|
| $B_1$ | + | - | - | - | + | - | + |
| $B_2$ | + | - | - | - | - | + (α) | + |
| $B_3$ | + | - | - | - | - | - | + |
| $B_4$ | + | - | - | - | + | - | + |
| $B_5$ | + | - | - | - | - | - | + |
| $B_6$ | + | - | - | - | - | - | + |
| $B_7$ | + | - | - | - | - | - | + |
| $B_8$ | + | - | - | - | - | + (α) | + |
| $B_9$ | + | - | - | - | - | - | + |
| $B_{10}$ | + | - | - | - | + | - | + |
| $B_{11}$ | + | - | - | - | - | - | + |
| $B_{12}$ | + | - | - | - | - | - | + |
| $B_{13}$ | + | - | - | - | - | - | + |
| $B_{14}$ | + | - | - | - | + | + (α) | + |
| $B_{15}$ | + | - | - | - | - | + (β) | + |
| $B_{16}$ | + | - | - | - | - | - | + |
| $B_{17}$ | + | - | - | - | + | - | + |
| $B_{18}$ | + | - | - | - | - | - | + |
| $W_1$ | + | - | - | - | - | - | + |
| $W_2$ | + | - | - | - | - | - | + |
| $W_3$ | + | - | - | - | + | - | + |
| $W_4$ | + | - | - | - | - | - | + |

Table 2: Continued

| Isolate code | Gram stain | Spore forming | Motility test | Oxidase | Catalase | Hemolysis | Starch hydrolysis |
|---|---|---|---|---|---|---|---|
| $W_5$ | + | - | - | - | - | - | + |
| $W_6$ | + | - | - | - | - | - | + |
| $C_1$ | + | - | - | - | - | - | + |
| $C_2$ | + | - | - | - | - | - | + |
| $C_3$ | + | - | - | - | - | - | + |
| $C_4$ | + | - | - | - | - | - | + |
| $C_5$ | + | - | - | - | - | - | + |
| $C_6$ | + | - | - | - | - | - | + |
| $C_7$ | + | - | - | - | - | - | + |
| $C_8$ | + | - | - | - | - | - | + |
| $C_9$ | + | - | - | - | + | - | + |
| $C_{10}$ | + | - | - | - | - | - | + |
| $C_{11}$ | + | - | - | - | - | - | + |
| $C_{12}$ | + | - | - | - | - | - | + |
| $C_{13}$ | + | - | - | - | - | - | + |
| $C_{14}$ | + | - | - | - | - | - | + |
| $C_{15}$ | + | - | - | - | - | - | + |
| $C_{16}$ | + | - | - | - | - | - | + |
| $C_{17}$ | + | - | - | - | - | - | + |
| $C_{18}$ | + | - | - | - | - | - | + |
| $C_{19}$ | + | - | - | - | - | - | + |
| $G_1$ | + | - | - | - | - | + (α) | + |
| $G_2$ | + | - | - | - | - | - | + |
| $G_3$ | + | - | - | - | - | - | + |
| $G_4$ | + | - | - | - | - | - | + |
| $G_5$ | + | - | - | - | - | - | + |
| $G_6$ | + | - | - | - | - | - | + |
| $K_1$ | + | - | - | - | - | - | + |
| $K_2$ | + | - | - | - | - | - | + |
| $K_3$ | + | - | - | - | - | - | + |
| $K_4$ | + | - | - | - | - | - | + |
| $K_5$ | + | - | - | - | + | - | + |
| $K_6$ | + | - | - | - | - | - | + |
| $K_7$ | + | - | - | - | - | - | + |
| $K_8$ | + | - | - | - | - | - | + |
| $K_9$ | + | - | - | - | - | - | + |
| $K_{10}$ | + | - | - | - | - | - | + |
| $F_1$ | + | - | - | - | - | - | + |
| $F_2$ | + | - | - | - | - | - | + |
| $F_3$ | + | - | - | - | - | - | + |
| $F_4$ | + | - | - | - | - | - | + |
| $F_5$ | + | - | - | - | - | - | + |
| $F_6$ | + | - | - | - | - | - | + |
| $F_7$ | + | - | - | - | - | - | + |
| $F_8$ | + | - | - | - | - | - | + |
| $F_9$ | + | - | - | - | - | - | + |
| $F_{10}$ | + | - | - | - | - | - | + |
| $F_{11}$ | + | - | - | - | - | - | + |
| $F_{12}$ | + | - | - | - | - | - | + |

Table 3: Acid production by different LAB isolates

| Isolate code | Δ pH at 4 h | Δ pH at 6 h | Isolate code | Δ pH at 4 h | Δ pH at 6 h |
|---|---|---|---|---|---|
| $B_1$ | 0.1±0.01 | 0.4±0.01 | $C_{13}$ | 0.3±0.01 | 0.6±0.01 |
| $B_2$ | 0.2±0.00 | 0.3±0.03 | $C_{14}$ | 0.2±0.00 | 0.3±0.04 |
| $B_3$ | 0.3±0.02 | 0.5±0.01 | $C_{15}$ | 0.4±0.04 | 0.8±0.03 |
| $B_4$ | 0.4±0.01 | 0.5±0.02 | $C_{16}$ | 0.3±0.01 | 0.5±0.04 |
| $B_5$ | 0.3±0.01 | 0.4±0.01 | $C_{17}$ | 0.3±0.02 | 0.4±0.02 |
| $B_6$ | 0.4±0.01 | 0.5±0.03 | $C_{18}$ | 0.5±0.04 | 0.5±0.01 |
| $B_7$ | 0.3±0.04 | 1.0±0.1 | $C_{19}$ | 0.3±0.02 | 0.6±0.03 |
| $B_8$ | 0.2±0.02 | 0.3±0.02 | $G_1$ | 0.1±0.03 | 0.4±0.04 |
| $B_9$ | 0.3±0.01 | 0.7±0.04 | $G_2$ | 0.7±0.02 | 1.2±0.02 |
| $B_{10}$ | 0.3±0.00 | 0.5±0.01 | $G_3$ | 0.3±0.01 | 0.7±0.04 |
| $B_{11}$ | 0.5±0.02 | 0.7±0.04 | $G_4$ | 0.4±0.04 | 0.9±0.01 |
| $B_{12}$ | 0.3±0.01 | 0.7±0.03 | $G_5$ | 0.3±0.02 | 0.5±0.01 |
| $B_{13}$ | 0.3±0.00 | 0.9±0.04 | $G_6$ | 0.2±0.03 | 0.3±0.03 |
| $B_{14}$ | 0.1±0.00 | 0.2±0.02 | $K_1$ | 0.4±0.01 | 0.7±0.04 |
| $B_{15}$ | 0.3±0.00 | 0.4±0.01 | $K_2$ | 0.3±0.04 | 0.8±0.02 |
| $B_{16}$ | 0.4±0.01 | 0.7±0.04 | $K_3$ | 0.3±0.03 | 0.5±0.04 |
| $B_{17}$ | 0.3±0.01 | 0.5±0.00 | $K_4$ | 0.4±0.02 | 0.6±0.01 |
| $B_{18}$ | 0.1±0.00 | 0.3±0.03 | $K_5$ | 0.3±0.04 | 0.5±0.03 |
| $W_1$ | 0.4±0.01 | 0.6±0.02 | $K_6$ | 0.5±0.03 | 0.7±0.02 |
| $W_2$ | 0.5±0.01 | 1.2±0.02 | $K_7$ | 0.6±0.04 | 0.8±0.04 |
| $W_3$ | 0.3±0.00 | 0.7±0.04 | $K_8$ | 0.3±0.02 | 0.7±0.02 |
| $W_4$ | 0.4±0.03 | 0.6±0.02 | $K_9$ | 0.5±0.04 | 0.7±0.03 |
| $W_5$ | 0.3±0.01 | 0.6±0.01 | $K_{10}$ | 0.5±0.03 | 0.8±0.04 |
| $W_6$ | 0.4±0.01 | 0.7±0.01 | $F_1$ | 0.3±0.01 | 0.5±0.03 |
| $C_1$ | 0.5±0.03 | 0.8±0.04 | $F_2$ | 0.2±0.04 | 0.3±0.04 |
| $C_2$ | 0.6±0.00 | 1.4±0.04 | $F_3$ | 0.5±0.02 | 0.7±0.01 |
| $C_3$ | 0.4±0.01 | 0.9±0.04 | $F_4$ | 0.1±0.00 | 0.3±0.04 |
| $C_4$ | 0.5±0.01 | 0.8±0.01 | $F_5$ | 0.2±0.04 | 0.4±0.02 |
| $C_5$ | 0.3±0.01 | 0.5±0.02 | $F_6$ | 0.4±0.02 | 0.5±0.01 |
| $C_6$ | 0.7±0.04 | 0.9±0.02 | $F_7$ | 0.3±0.03 | 0.6±0.04 |
| $C_7$ | 0.4±0.01 | 0.6±0.03 | $F_8$ | 0.3±0.04 | 0.4±0.02 |
| $C_8$ | 0.3±0.02 | 0.7±0.01 | $F_9$ | 0.1±0.00 | 0.4±0.01 |
| $C_9$ | 0.5±0.01 | 0.6±0.04 | $F_{10}$ | 0.2±0.02 | 0.3±0.04 |
| $C_{10}$ | 0.0±0.00 | 0.2±0.03 | $F_{11}$ | 0.5±0.04 | 0.7±0.02 |
| $C_{11}$ | 0.3±0.01 | 0.7±0.02 | $F_{12}$ | 0.4±0.03 | 0.6±0.03 |
| $C_{12}$ | 0.4±0.03 | 0.8±0.01 | | | |

In this work, all physiological and biochemical tests were similar as those obtained by Sharpe (1979), Axelsson (1998) and Cullimore (2000) who described their LAB as being Gram-positive, catalase -ve, oxidase -ve, non spore former, non motile, hydrolyze starch and have different hemolytic activity.

However, characteristics observed in the properties of the strain $B_1$, $B_4$, $B_{10}$, $B_{14}$, $B_{17}$, $W_3$, $C_9$ and $K_5$ as being catalase +ve (very weak) was contradictory to the above investigators. These pseudocatalases cultures of LAB were confirmed by Abriouel *et al.* (2004). Also, Whittenbury (1960) found two types of catalase +ve of LAB isolates and referred them as catalase-like activity in LAB.

Table 3 revealed the acidifying activity of isolated LAB strains. The change in pH (Δ pH) was ranged from 0.2 to 1.2 after 6 h incubation. The cultures were described as fast, moderately or slow

Table 4: Screening of the recovered bacterial isolates for antimicrobial activity against test pathogens

| | Mean diameter of inhibition zone (mm) ±SD | | |
|---|---|---|---|
| Isolate code | *E. coli* | *S. aureus* | *C. albicans* |
| $B_1$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_2$ | 12.0±0.5 | 0.0±0.0 | 10.0±0.4 |
| $B_3$ | 0.0±0.0 | 10.0±0.3 | 0.0±0.0 |
| $B_4$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_5$ | 0.0±0.0 | 0.0±0.0 | 12.0±0.2 |
| $B_6$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_7$ | 18.0±0.3 | 18.0±0.4 | 14.0±0.3 |
| $B_8$ | 0.0±0.0 | 14.0±0.3 | 0.0±0.0 |
| $B_9$ | 9.0±0.5 | 0.0±0.0 | 0.0±0.0 |
| $B_{10}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_{11}$ | 0.0±0.0 | 12.0±0.5 | 0.0±0.0 |
| $B_{12}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_{13}$ | 9.0±0.5 | 0.0±0.0 | 0.0±0.0 |
| $B_{14}$ | 0.0±0.0 | 0.0±0.0 | 11.0±0.0 |
| $B_{15}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_{16}$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.2 |
| $B_{17}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $B_{18}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $W_1$ | 10.0±0.2 | 0.0±0.0 | 0.0±0.0 |
| $W_2$ | 16.0±0.5 | 13.0±0.3 | 12.0±0.4 |
| $W_3$ | 12.0±0.3 | 10.0±0.2 | 0.0±0.0 |
| $W_4$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $W_5$ | 13.0±0.4 | 0.0±0.0 | 0.0±0.0 |
| $W_6$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_1$ | 0.0±0.0 | 10.0±0.1 | 0.0±0.0 |
| $C_2$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_3$ | 10.0±0.1 | 0.0±0.0 | 0.0±0.0 |
| $C_4$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_5$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.3 |
| $C_6$ | 0.0±0.0 | 0.0±0.0 | 9.0±0.0 |
| $C_7$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_8$ | 10.0±0.2 | 0.0±0.0 | 0.0±0.0 |
| $C_9$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_{10}$ | 0.0±0.0 | 0.0±0.0 | 11.0±0.0 |
| $C_{11}$ | 0.0±0.0 | 8.0±0.3 | 0.0±0.0 |
| $C_{12}$ | 17.0±0.2 | 16.0±0.2 | 14.0±0.0 |
| $C_{13}$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.0 |
| $C_{14}$ | 13.0±0.5 | 0.0±0.0 | 0.0±0.0 |
| $C_{15}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_{16}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_{17}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $C_{18}$ | 0.0±0.0 | 0.0±0.0 | 12.0±0.2 |
| $C_{19}$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.4 |
| $G_1$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $G_2$ | 17.0±0.3 | 16.0±0.3 | 12.0±0.3 |
| $G_3$ | 13.0±0.2 | 0.0±0.0 | 0.0±0.0 |
| $G_4$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

Table 4: Continued

| Isolate code | Mean diameter of inhibition zone (mm) ±SD | | |
|---|---|---|---|
| | *E. coli* | *S. aureus* | *C. albicans* |
| $G_5$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $G_6$ | 9.0±0.1 | 0.0±0.0 | 0.0±0.0 |
| $K_1$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $K_2$ | 13.0±0.3 | 0.0±0.0 | 0.0±0.0 |
| $K_3$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $K_4$ | 11.0±0.5 | 0.0±0.0 | 0.0±0.0 |
| $K_5$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $K_6$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.2 |
| $K_7$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $K_8$ | 10.0±0.3 | 10.0±0.3 | 0.0±0.0 |
| $K_9$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $K_{10}$ | 9.0±0.5 | 10.0±0.2 | 0.0±0.0 |
| $F_1$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_2$ | 9.0±0.6 | 0.0±0.0 | 0.0±0.0 |
| $F_3$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_4$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_5$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.3 |
| $F_6$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_7$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_8$ | 0.0±0.0 | 0.0±0.0 | 10.0±0.3 |
| $F_9$ | 10.0±0.5 | 8.0±0.5 | 0.0±0.0 |
| $F_{10}$ | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| $F_{11}$ | 0.0±0.0 | 10.0±0.1 | 0.0±0.0 |
| $F_{12}$ | 10.0±0.3 | 0.0±0.0 | 0.0±0.0 |

*MRSA: *F. oxysporum* and *A. niger* were not presented as they gave -ve results

acidifying as ΔpH was ranged from 0.8 to 1.2, from 0.4 to 0.7 or from 0.2 to less than 0.4, respectively (Ayad *et al*., 2004). These results are in agreement with those of El-Soda *et al*. (2003) and Durlu-Ozkaya *et al*. (2001) who reported that LAB strains were differed in their ability to reduce the pH of medium after 6 h.

**Screening of isolates for antimicrobial activity:** All the bacterial isolates (71) which were recovered from different LAB habitats, were screened for their antimicrobial activity against test pathogens *Escherichia coli, Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans, Aspergillus niger* and *Fusarium oxysporum*. Forty of these isolates were antagonistic for test bacteria (*E. coli, S. aureus*) and *C. albicans* but none of them could exert antagonistic activity against MRSA, *A. niger* and *F. oxysporum* which were resistant (Table 4). The highest inhibition was achieved by isolates $B_7$, $W_2$, $C_{12}$ and $G_2$. Maximum inhibition zone was obtained by isolate $B_7$ where 18 mm were recorded for both *E. coli* and *S. aureus* and 14 mm for *C. albicans*. Isolate $W_2$ gave inhibition zones of 16, 13 and 12 mm with *E. coli, S. aureus* and *C. albicans*, respectively. Isolates $C_{12}$ and $G_2$ achieved the same inhibition zones with *E. coli*.

In this respect, many studies have focused on antibacterial compounds secreted by LAB which inhibit the main undesirable poultry pathogens *Salmonella enterica* and *Escherichia coli* (Pascual *et al*., 1999; Ashraf *et al*., 2005; Ma *et al*., 2009). Batish *et al*. (1989) found that only 5

LAB cultures out of 19, using a well diffusion assay, were able to inhibit mould growth. None, however, were able to inhibit any of the 6 yeast species studied (*Saccharomyces cerevisiae* strains 522, SCB, SC-1; *Saccharomyces fragilis* strain 3465; *Candida guillermondia* strain 3124 and *Rhodotorula glutinis* strain RG).

Research about fungal inhibition by LAB and the compounds produced by these bacteria is still novel (Maganusson and Schnurer, 2001). However, Gourama and Bullerman (1995) found that *Lactobacillus* spp inhibit *A. flavus* spore viability, spore germination, growth and aflatoxin production.

Interestingly, the present result revealed that not all the 40 species showed the same antagonistic activity against all test pathogens i.e., one bacterial isolate like $B_2$ (Table 4) gave activity against *E. coli* and *C. albicans* but not *S. aureus* and $B_8$ exerted activity against *S. aureus* but not *C. albicans* or *E. coli* and so on. Similar results were obtained by Jay (2000) and Adetunji and Adegoke (2007).

**Identification of bacteria up to the genus level:** In the present study, identification of the 71 isolates, up to the genus level, revealed that they belonged to the genera *Lactobacillus* (27 isolates :$B_1$, $B_4$, $B_6$, $B_9$, $B_{10}$, $B_{12}$, $B_{16}$, $B_{17}$, $B_{18}$, $W_3$, $W_5$, $C_1$, $C_6$, $C_{10}$, $C_{11}$, $G_2$, $G_4$, $G_6$, $K_2$, $F_1$, $F_3$, $F_4$, $F_5$, $F_6$, $F_7$, $F_9$, $F_{11}$), *Streptococcus* (12 isolates: $B_2$, $B_5$, $B_7$, $B_8$, $B_{13}$, $B_{14}$, $B_{15}$, $W_4$, $G_1$, $G_5$, $K_5$, $K_8$), *Pediococcus* (11 isolates $W_1$, $W_6$, $C_2$, $C_{12}$, $C_{13}$, $C_{14}$, $C_{18}$, $C_{19}$, $K_6$, $F_2$, $F_{12}$), *Leuconostoc* (16 isolate :$C_3$, $C_4$, $C_7$, $C_8$, $C_9$, $C_{15}$, $C_{16}$, $C_{17}$, $G_3$, $K_1$, $K_2$, $K_4$, $K_7$, $K_9$, $F_8$, $F_{10}$) and *Lactococcus* (5 isolates: $B_3$, $B_{11}$, $W_2$, $C_5$, $K_{10}$).

The isolates (40) which gave antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* were further identified up to the species level using API 50CH kits. These species were included in Table 5. The highly promising isolates which achieved the greatest inhibition against the test pathogenic organisms were *Streptococcus salivarius* (isolate $B_7$), *Lactococcus lactis* ($W_2$), *Pediococcus*

Table 5: Identification of isolates by API 50 CH

| Isolate code | Name of species | Similarity (%) | Isolate code | Name of species | Similarity (%) |
|---|---|---|---|---|---|
| $B_2$ | Streptococcus mitis | 95 | W3 | Lactobacillus plantarum | 98 |
| $B_3$ | Lactococcus lactis | 86 | W5 | Lactobacillus lactis | 90 |
| $B_5$ | Streptococcus sobrinus | 80 | C1 | Lactobacillus plantarum | 96 |
| $B_7$ | Streptococcus salivarius | 90 | C3 | Lactococcus lactis | 80 |
| $B_8$ | Streptococcus mitis | 92 | C5 | Lactococcus lactis | 88 |
| $B_9$ | Lactobacillus plantarum | 92 | C6 | Lactobacillus lactis | 91 |
| $B_{11}$ | Lactococcus lactis | 93 | C8 | Leuconostoc mesenteroides | 90 |
| $B_{13}$ | Streptococcus ratius | 75 | C10 | Lactobacillus plantarum | 95 |
| $B_{14}$ | Streptococcus mitis | 76 | C11 | Lactobacillus helveticus | 88 |
| $B_{16}$ | Lactobacillus plantarum | 87 | C12 | Pediococcus pentosaceus | 94 |
| $W_1$ | Pediococcus pentosaceus | 88 | C13 | Pediococcus acidilactici | 90 |
| $W_2$ | Lactococcus lactis | 95 | C14 | Lactococcus lactis | 84 |
| $C_{18}$ | Pediococcus pentosaceus | 89 | K6 | Pediococcus pentosaceus | 90 |
| $C_{19}$ | Pediococcus pentosaceus | 97 | K10 | Lactococcus lactis | 88 |
| $G_2$ | Lactobacillus plantarum | 96 | F2 | Pediococcus pentosaceus | 93 |
| $G_3$ | Leuconostoc lactis | 95 | F5 | Lactobacillus plantarum | 92 |
| $G_6$ | Lactobacillus curvatus | 90 | F8 | Leuconostoc mesenteroides | 95 |
| $K_2$ | Lactobacillus plantarum | 79 | F9 | Lactobacillus lactis | 90 |
| $K_4$ | Leuconostoc mesenteroides | 96 | F11 | Lactobacillus plantarum | 95 |
| $K_8$ | Lactococcus lactis | 99 | F12 | Pediococcus pentosaceus | 91 |

*pentosaceus* ($C_{12}$) and *Lactobacillus plantarum* ($G_2$). Abdelgadir *et al.* (2001) studied the microbiology of local cow's milk and revealed the presence of high count of lactococci and lactobacilli.

From the above discussion it could be suggested that, lactic acid bacteria represent a commercial potential source of much-needed new natural antimicrobial agents, which could be promising for management of the present test pathogens.

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