ABSTRACT

The increasing demand of consumers who look for natural safe products and associated health risks of chemically treated and refined goods food products led to the introduction of alternative technologies for preservation and maintenance of dietary freshness. One of such preservation technology requires the use of Lactobacilli as starter culture for the preservation of food matrices planned. The present study was designed to evaluate sweet whey low-cost by-product of dairy manufacture as a substrate for the selection of Lactobacilli with proven antibacterial activity to be used as biopreservation for fermented dairy product. Human population has used dairy products, including milk, as a source for Lactic acid bacteria (LAB). The positive impact of the given bacteria as a diet supplement has been of concern to researchers. The target of this study is to examine Lactobacillus species isolated from dairy products i.e. raw milk alongside cheese, with potential activities. Using the Sweet whey as growth medium for Lactic acid bacteria. Also, tested of antibacterial activities. Sweet whey (sw) proved to be suitable medium alternative to the expensive commercial De Man-Regosa-Sharp medium for the growth and production of antibacterial substances by isolated lactic acid bacteria isolates. Therefore, was used throughout the present work. A number of 32 isolates were obtained from raw cow milk (16 isolates), goat milk (9 isolates) and cottage cheese (7 isolates) using the specific De Man-Regosa-Sharp medium (MRS) for the isolation of LAB, these isolates were identified up to genus as strains of Lactobacillus spp. Among 32 isolates of Lactobacillus spp., there are twenty four isolates antagonized of the all seven tested pathogenic bacteria. The screened Lactobacillus spp. isolates were characterized and selected the best bio agent isolate against pathogens was identified using 16s DNA gene to Lactobacillus brevis. This strain was resistant to NaCl (2-6.5 %), produces dioxide carbon (CO₂) and showed good growth in different temperature (10°, 15, 45°C) and fermented of many sugars. As per the outcomes shown herein, the strain in question was thought to be antimicrobial bacterium produced a variety of metabolites, including butyric, formic, lactic, citric, and sorbic acid. The given set of activities adds to microbiological safety by helping control microorganism growth, and inhibiting pathogenic bacteria by using the lower-cost sweet whey. Therefore, more detailed work on isolating and characterizing antibacterial bacteria from the locally produces dairy items, and augmenting their growth could be needed for the creation of bio-preservation foods.

Keywords: Lactobacilli, Dairy products, bio-preserve, antibacterial. Bioactive gradients. 16s DNA gene, PCR

INTRODUCTION

In recent years, live Lactic acid bacteria and non-pathogenic micro-organisms that prove beneficial for health have amassed attention worldwide
because of their commercial aspects. Lactic acid bacteria are typically deployed when fermented food is being created and are considered safe for veterinary and medical use. Common dairy items that have been in use for several years are the principal source of major antimicrobial bacteria. According to the Agricultural Science and Technology Board, food-borne microbial pathogens contribute to more than 6.5-33 million cases of disease, and about 9,000 deaths annually. The main food items that cause health issues are seafood, dairy, poultry and meat. In majority of the cases, the pathogens at play include issues like *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. As well as producing Lactic acid bacteria, which impedes the production of certain microorganisms, allows them to grow within the intestinal tract (Murry et al. 2004). Lactic acid bacteria are producing a many of antibacterial metabolites, i.e., organic acids, hydrogen peroxide, acetoain, diacetyl, and bacteriocins. These activities contribute to microbiological health by regulating other microorganism growth and pathogenic bactera inhibition (Osuntoki and Korie, 2010). Lactobacilli are an important part of our intestinal microflora, and their role in the general state of human health is being studied (Guarnieri et al. 2012). The *Lactobacillus* spp. genus is one of the major groups of lactic acid bacteria used in the fermentation of food, and is therefore of great economic importance.

The taxonomy of the group Lactobacilli group has undergone essential changes that have created confusion in recent years (Pennacchia et al 2004). A variety of studies have been carried out to define and classify LAB. This includes conventional biochemical testing, i.e. carbohydrate fermentation trends through use of commercially sold kits, physiological experiments (Axelsson 1993), and more nuanced molecular biology methods. Lactobacilli were not associated with disease in general and were seen as non-pathogenic parts of the urogenital and intestinal flora (Ali 2012). Lactobacilli has a role to play in the healthy state of the gastrointestinal ecosystem through antagonistic engagement with the under-focus pathogenic bacteria. *Lactobacillus* species producing antibacterial compounds, including hydrogen peroxide (*H₂O₂*), Lactic and other organic acids, and bacteriocins, perform regulatory processes. The goal of this investigation is therefore to use the waste from dairy by-products, including Sweet Whey at the time that the growth medium of lactobacillus spp. To produce bio-preservatives and select the best lactobacillus strain as antibacterial against pathogenic bacteria and optimize their growth by means of static analysis (pH, NaCl stress, incubation period, inoculum size and incubation temperature).

**MATERIALS AND METHODS**

**Samples collection**: The different samples, cottage cheese, goat milk and cow milk were collected from the Giza province's local areas. They were transported to the refrigerated laboratory (4°C), and were immediately analyzed.

**Culture sources**: *Lactobacillus helveticus* strain ATCC15009 to compare with LAB isolates and Seven foodborne bacteria and pathogens were kindly collected from the Ain-Shams Univ., Fac. of Agric., Microbiology Research Center (MIRCEEN) in Cairo, Egypt. These strains were *Bacillus cereus* ATCC33018, *Staphylococcus aureus* ATCC29737, *Listeria monocytogenes* ATCC19115 (as Gram-positive bacteria), *Escherichia coli* O157: H1 ATCC25922, *Salmonella typhimurium* ATCC25566, *Pseudomonas aeruginosa* ATCC27853, and *Shigella sonnei* ATCC25931 (as Gram negative bacteria). These strains have been used and activated for the assessment of antibacterial activity of tested lactic acid bacteria.

**Isolation of Lactic acid bacteria**: Ten mL of raw milk per sample and 10 g of cottage cheese have been homogenized with 90 mL of MRS medium sterile broth. Make sufficient serial dilutions of up to 10^-8 by adding 1 mL in 9 mL of the diluent (Lairini et al 2014).

One mL of every sample dilution was added to sterile Petri Dishes with MRS agar specific medium (De Man et al 1960) and 48-72 h incubation at 37°C. Repeated streaking on MRS agar medium purified the selected colonies (Ounine et al 2004). The pure isolates of lactic acid bacteria were stored at 4°C and regenerated every four weeks to maintain those (Badis et al 2005).

**Identification of Lactic acid bacteria isolated to genus level**

**Macroscopic analyses**: Macroscopic characters of the cell shape, were examined by light microscope at 1000 X and colonies of lactic acid bacteria is studied by a binocular loupe (Motic) in order to describe their morphology; color, surface, elevation, board, aspect and opacity using a microscope (Motic) linked to a computer and a camera to take pictures with the objective 100.
physiological and biochemical tests

Categorization of isolates was based on Gram staining and catalase test. Only the strains Gram-positive and catalase negative were retained. Further identification was carried out using the ability to expand to a tolerance of 6.5 percent NaCl at different temperatures of 10°C, 15°C and 45°C, and Gas production.

Incubation temperatures: The growth test at different temperatures consists of inoculating the young cultures of LAB isolates in tubes containing the medium of MRS broth, then incubated at 10°C 15°C, and 45°C for 72 h. LAB growth indicates how tolerant these temperatures are.

Catalase test: this activity was outlined with the addition of 3% (v/v) (sigma) hydrogen peroxide (H2O2) into cultivated colonies, which has been outlined under (Whittenbury, 1964).

Sensitivity to salt: The isolates were tested for tolerance to 6.5% (w/v) NaCl concentrations in MRS broth medium. The tubes were inoculated with young cultures and then incubated at 37°C /48 h.

Gas production: The homo or hetero fermentative character of the isolates is studied basing their ability or not ability to produce Carbon dioxide gas (CO2). The test was studied using inverted Durham tubes on MRS broth medium (Khedid et al 2009).

Carbohydrate fermentation: Carbohydrate fermentation of isolates by using Api 32 STREP (Bio Mérieux, France) using glucose, sucrose, xylose, sorbitol and lactose according to the manufacturer’s instructions (Ismaili et al 2016), and for 48 hours Api STREPS had been incubated at 37°C.

Preparation of sweet whey medium: As stated, growth of LAB isolates in the sweet whey medium was achieved (Huang et al 2016a). The sweet whey medium was prepared by dissolving the sweet whey powder in distilled water to produce 10 percent of the medium with final total solid content (TS, w/w). This culture medium then was autoclaved at 100°C for 20 min for 3 days before inoculation, respectively. For inoculation, put (1% inoculum size) from young cultures and then incubated at 37°C for 48 h.

Preparation of Cell free supernatant (CFS) of isolates: CFS of all the tested isolates was prepared (6000 rpm/30 min/4°C) of actively growing Lactic acid bacteria sub-cultured overnight, followed by aseptic collection of supernatants. The collected CFS was filter-sterilized by passing through a 0.2 μm PVDF Whatman sterile Uniflo pore size filter (Nisha and Krishnamoorthy, 2018).

Evaluation of antibacterial activity of isolates by Disc diffusion method: By testing the antimicrobial activity of LAB isolates which are grown on a sweet whey medium against seven pathogenic bacteria and using commercial strain (L. helveticus ATCC15009) for comparing by using the method of disc diffusion as described by (Sandra et al 2012). These assays were conducted in triplicate, the plates were poured with 20 ml of Nutrient Agar, the pathogenic strains were spread over the nutrient agar surface. Before placing the cell-free supernatant (CFS) impregnated paper discs on the plates, the agar plates inoculated with indicator organism were incubated for 1 h. For the screening of antimicrobial activity, 3 sterile paper discs with a diameter of 6 mm were saturated with 10 μl of the filtered supernatant of tested isolate on the surface of the agar plate inoculated by indicator strains. At 37°C /24 hours the plates were incubated. Discs dipped in non-inoculated sterile medium acted as control. Inhibition zones is measured in millimeters with calipers (Lleó et al 1998; Mir-hoseini 2004).

Antimicrobial characterization: LAB isolates and L. helveticus were examined for the production of antibacterial substances using the technique of disc diffusion. For different assays the grown culture on sweet whey broth was divided into equal fractions. 10 ml of supernatant was treated with 1 mg/ml of catalase for hydrogen peroxide assayed and incubated for 2h at 37°C. To exclude the antibacterial effect of organic acids, 10 ml of supernatant was adjusted to pH 6.5 ± 0.1 using 1 NaOH. The WDM was then tested for antagonistic activity against indicator bacteria after treatment and neutralization of cell-free supernatants as described above (Ghalfi et al 2006). Inhibition zone was noted after 24 h of incubation at 37°C.

Selection of the most active Lactobacillus spp. isolates: Selection of the most active isolates as antimicrobial against of the pathogenic strains by pasteurization at 72°C / 15 second for the supernatant and testing of antimicrobial activity by disc diffusion then measuring inhibition zone and assayed for residual activity.

Definition selected Lactobacillus isolate by 16s DNA gene

DNA Extraction: The supernatant was discarded and the pelt in the saline buffer was suspended.
μl suspended bacteria were placed in 2ml Ependorf tube and 95 μl of water, 95 μl of solid tissue buffer (blue) and 10 μl of proteinase K were added. The mixture was incubated for 2 hours at 55°C, and centrifuged for 1 minute at 12,000 x g. The aequous supernatant has been transferred to a purified tube (300 μl). 600 ul Combined Genomic Binding Buffer. The mixture was transferred for 1 minute in a Collection Tube Centrifuge at a Zymo-Spin™ IIC-XL column (12,000 x g). With the flow through discard the collection tube400 μl DNA Pre-Wash Buffer added to Collection Tube and centrifuged at (12,000 x g) for 1 minute. 700 μl of g-DNA Wash buffer was added and centrifuged for 1 minute at (12,000 x g). Empty the tube of the set. Add 200 μl of g-DNA Wash buffer was added and centrifuged for 1 minute at (12,000 x g). 3 μl of elution buffer was added and incubated for 5 minutes, then centrifuged for 1 minute at (12,000 x g). PCR reaction setup: 25 μL My Taq Red Mix (PCR mix with my base of Taq, PCR buffer and DNA T); 8 μL DNA Template; 1 μL (20 Pico mol) Forward Primers; 1 μL (20 Pico mol) Reverse Primers; 15 μL Nuclease Free Water. PCR reaction was performed in a thermal cycler condition at 35 cycles of step temperature, each cycle included initial denaturation at 94°C for 6 minutes, Denaturation at 94°C for 45 s; finishing at 56°C for 45 s Extension at 72°C for 1 minute and finishing at 72°C for 5 minutes.

The sequencing performed on GATC Company to the PCR product using ABI 3730xI DNA se-
quencer using forward and reverse primers. It is only by combining traditional Sanger technology with the new 454 technology that genomes can now be sequenced and analyzed in half the usual project time, with a significant reduction in the number of coatings and lacunae. Additionally, significant cost advantages now make genome sequencing accessible to the research community with the 454 technology.

Analysis of cell-free supernatant (CFS) by HPLC

Analysis of HPLC selected strain organic acids in cell-free supernatant (CFS), as discerned by (Hu et al 2019). The pH curve is 3.7 and reaches the end of the log phase at 24 hr. The fermented broth was used to separate. The CFS was obtained under cooling by centrifuging 20 ml of the fermented broth at 6,000 rpm for 30 min. The CFS has been applied to 1 ml of ammonium di hydrogen phosphate buffer, 3% of methanol, homogenized and Centrifuged at 14,000 g for 15 min and 0.22-μm filter (Nylon; Re-
phiLe Bioscience) was filtered under the cooling process into HPLC amber vials Unfermented MRS broth was tested for controls under similar conditions. The used pure reagents, L(+)-tartaric acid and L(-) (malic acid. First, seven common organic acids were identified as standard curves: oxalic acid, tartaric acid, malic acid, lactic acid, citric acid, acetic acid and succinic acid. At the same time it was determined the retaining time of 8 organic acids. The organic acids were detected and quantified using a Photodiode Array Detector (SPD-M20A) Shimadzu Nexera LC system.

A column of C18 (250/4.6 mm I.D., 5 μm; Teknokroma) was used for chromatographic separation.

The organic acids were eluted using H2O with 11.5 % ammonium dihydrogen phosphate (solvent A) and methanol (solvent B). Before use, both solvents were filtered and degassed 0.22-μm. Isocratic elution: solvent A 97 % and solvent B 3 %. The flow rate was set at 0.7 ml / min, the temperature set at 25 °C, and the volume was injected at 10 μl. For UV organic acids were detected at a wavelength of 210 nm. Cell-free supernatant (CFS) analysis by HPLC mode at the Regional Mycology and Biotechnology Centre, Cairo, Egypt.

Statistical analysis: Statistical analysis was done by two designs (Taguchi design and Central com-
position design (CCD)).

RESULTS AND DISCUSSION

Isolation of Lactobacillus spp.

Thirty two of Lactic acid bacteria were isolated from different sources, 7 isolates named, K1 to K7) isolated from cottage cheese, 9 isolates named, G1 to G9) isolated from goat milk, and 16 isolates. (named, C1 to C16) isolated from cow milk. All isolates were rod shape and Gram positive stain.

Physiological and biochemical characters

These isolates and Lactobacillus helveticus strain did not produce CO2 from glucose fermentation indicating that they are homo fermentative except isolate C7 is hetero fermentative due to produce CO2. All isolates and Lactobacillus helveticus strain ferment glucose, lactose, Sucrose, and Xylose, most of them ferment Sorbitol. They grew at 10°C, but some of them didn’t grow at this temperature, they were (C1, C2, C3, C4, C5, and C6) and the isolates weren’t grown at 15°C (C1, C3, C4, and C5), but all isolates were grown at 45°C. All isolates grew at 2.4% NaCl, while some isolates grew at 6.5% NaCl and some of them didn’t grow like (C1, C3, C4, and C6). Using these characteristics all isolates

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were identified as members of the genus \textit{Lactobacillus} spp. according to Bergey's Manual of determinative Bacteriology (Table 1).

\textbf{Antibacterial activity}

Evaluation of 32 \textit{Lactobacillus} spp. isolates and \textit{Lactobacillus helveticus} strain against 7 pathogens on broth sweet whey medium by disc diffusion assay by (Sandra et al 2012) was estimated. They were produced with differed strong inhibition zones against 7 pathogens. It was found \textit{Lactobacillus helveticus} strain and 28 out of 32 isolates except (K2, G5, G6, G7) were active as antibacterial against \textit{Bacillus cereus}, \textit{Lactobacillus helveticus} strain and 29 out of 32 isolates except (G5, G6, G7) were active as antibacterial against \textit{Staphylococcus aureus}, \textit{Lactobacillus helveticus} strain and 27 out of 32 isolates except (K1, K2, K7, G5, G6) were active as antibacterial against \textit{Listeria monocytogenes}, \textit{Lactobacillus helveticus} strain and 31 out of 32 isolates except only (G6) were active as anti \textit{E. coli}, \textit{Lactobacillus helveticus} strain and 28 out of 32 isolates except (G5, G6, G7, C13) were active as antibacterial against \textit{Pseudomonas aeruginosa}, \textit{Lactobacillus helveticus} strain and all 32 isolates were active as antibacterial against \textit{Shigella sonnei} and \textit{Lactobacillus helveticus} strain and 29 out of 32 isolates except (G5, G6, G7) were active as antibacterial against \textit{Salmonella typhimurium}. Antagonistic activity of LAB isolates against all 7 pathogenic bacteria, with different efficiency are presented in (Table 2).

\textbf{Characterization of antimicrobial substances}

\textbf{Total acidity activity:} The antibacterial activity of 24 selected \textit{Lactobacillus} spp. Isolates and \textit{Lactobacillus helveticus} strain before acid neutralized was appeared because of its organic acid production but disappeared after acid neutralized. On the other hand, not produced bactriocin and H$_2$O$_2$, where after eliminated effect of H$_2$O$_2$.

\textbf{Selection of the most active isolates}

After the pasteurization, only 11 isolates from 24 (11/24) were not sensitivity for the pasteurization, but only 5 isolates antagonized the 7 pathogens showing- highest residual activity after pasteurization. There strains were K5 and K6 from Cottage cheese, C3 and C7 from cow milk and G9 from goat milk (Table 3). Of this C7 isolate showed the relatively highest residual activity against \textit{Staphylococcus aureus} (87.8%), \textit{E. coli} (72.2%), \textit{Pseudomonas aeruginosa} (92.6%) and \textit{Shigella sonnei} (91.9%) as compared with the remainder four isolates.

\textbf{Molecular characters of \textit{Lactobacillus} spp.}

\textbf{Total DNA extraction:} Total DNA was extracted from \textit{Lactobacillus} spp. Gel electrophoresis and a UV spectrophotometer verified the quality and quantity of the filtered DNA. The \textit{Lactobacillus} spp. concentration was 75 μg/0.5 gm and the purity of the total DNA measured for \textit{Lactobacillus} spp. by an A260/280 absorbance ratio (1.6) suggesting high yield and purity of the extracted DNAs.

\textbf{16s DNA Gene Amplification:} \textit{Lactobacillus} spp. / 16s The DNA gene was amplified from \textit{Lactobacillus} spp. extracted DNAs. Use of the resulting PCR technique \textit{Lactobacillus} spp. / DNA mixed directly with the PCR reaction mixture, taq DNA polymerase and (sense and antisense) primers. Electrophoresis study of the amplified 16s DNA gene corresponding to the 16s DNA gene in the C-terminal region. The size of the PCR product amplified from \textit{Lactobacillus} spp. /DNA was estimated by comparing its electrophoretic mobility with those of standard DNA marker as shown in (Fig. 2). The amplified cDNA was in the expected size calculated (1100 bp) from the positions of sense primers.

\textbf{Nucleotide sequence analysis}

The PCR-amplified partial nucleotide sequence fragmented the 16s DNA gene of \textit{Lactobacillus} spp. isolate in the C-terminal region. Determining the relationship with other recommended \textit{Lactobacillus} spp. strains registered with GenBank. The 16s DNA gene sequence was performed using PCR produced when the specific (downstream) primers of \textit{Lactobacillus} spp. for 16s RNA gene were used. Nucleotides were found to be 1049 bp corresponding to the C-terminal region of 16s RNA gene (Fig. 3).

\textbf{Bioinformatics analysis of molecular data}

The partial nucleotide sequence of 16s RNA gene was aligned with 21 \textit{Lactobacillus} isolates with (Table 4). The partial sequence was multiple-aligned with the claulstral W program with minor manual adjustments (Fig. 4). The distances of nucleotides between isolates ranged from 0.004 to 0.064 (Table 4). The lower values for the \textit{Lactobacillus paucivorans} isolate pairs (NR 116943 1) were recorded. For 20 isolates, the greater nucleotide distance values were reported. A phylogenetic tree of 16s RNA gene revealed 98.43 % of similarity to \textit{Lactobacillus brevis} (Accession no NR_116238 1) (Fig. 4).
Table 1. Physiological and biochemical characters: of Isolated *Lactobacillus* spp.

| Source      | Isolate no. | Fermentation of sugars | Gas temperature | NaCl  |
|-------------|-------------|------------------------|-----------------|-------|
|             |             | G Lac Xy Su So         | CO₂ 10°C 15°C 45°C 2% 4% 6.5% |       |
| Cottage cheese | K1 | + + + + + + | - | + + + + + + |
|      | K2 | + + + + + + | - | + + + + + + |
|      | K3 | + + + + + + | - | + + + + + + |
|      | K4 | + + + + + + | - | + + + + + + |
|      | K5 | + + + + + + | - | + + + + + + |
|      | K6 | + + + + + + | - | + + + + + + |
|      | K7 | + + + + + + | - | + + + + + + |
|      | G1 | + + + + + + | - | + + + + + + |
|      | G2 | + + + + + + | - | + + + + + + |
|      | G3 | + + + + + + | - | + + + + + + |
|      | G4 | + + + + + + | - | + + + + + + |
| Goat Milk  | G5 | + + + + + + | - | + + + + + + |
|      | G6 | + + + + + + | - | + + + + + + |
|      | G7 | + + + + + + | - | + + + + + + |
|      | G8 | + + + + + + | - | + + + + + + |
|      | G9 | + + + + + + | - | + + + + + + |
|      | C1 | + + + + + + | - | + + + + + + |
|      | C2 | + + + + + + | - | + + + + + + |
|      | C3 | + + + + + + | - | + + + + + + |
| Cow Milk   | C4 | + + + + + + | - | + + + + + + |
|      | C5 | + + + + + + | - | + + + + + + |
|      | C6 | + + + + + + | - | + + + + + + |
|      | C7 | + + + + + + | + | + + + + + + |
|      | C8 | + + + + + + | - | + + + + + + |
|      | C9 | + + + + + + | - | + + + + + + |
|      | C10| + + + + + + | - | + + + + + + |
|      | C11| + + + + + + | - | + + + + + + |
|      | C12| + + + + + + | - | + + + + + + |
|      | C13| + + + + + + | - | + + + + + + |
|      | C14| + + + + + + | - | + + + + + + |
|      | C15| + + + + + + | - | + + + + + + |
|      | C16| + + + + + + | - | + + + + + + |
| *L. helveticus* | | | | |

(G): Glucose (Lac): Lactose (Xy): Xylose (Su): Sucrose (So): Sorbitol
Table 2. The diameter (mm) of inhibition zone of isolates against tested pathogens

| Source       | Isolate no. | B. cereus | Staph. aureus | L. monocyogenes | E. coli | Ps. aeruginosa | Shigella sonnei | S. typhimurium | Antagonized No. |
|--------------|-------------|-----------|---------------|-----------------|--------|----------------|-----------------|----------------|-----------------|
| Cottage cheese | K1          | 9.00      | 8.00          | 0.00            | 20.00  | 12.00          | 19.00           | 8.00           | 6               |
|              | K2          | 0.00      | 9.60          | 0.00            | 22.00  | 12.00          | 21.00           | 14.00          | 5               |
|              | K3          | 12.80     | 10.30         | 13.00           | 20.00  | 16.00          | 22.00           | 14.00          | 7               |
|              | K4          | 13.50     | 9.00          | 21.00           | 23.00  | 13.00          | 16.00           | 11.00          | 7               |
|              | K5          | 15.00     | 13.00         | 16.30           | 25.00  | 12.60          | 27.00           | 19.00          | 7               |
|              | K6          | 17.00     | 12.60         | 19.60           | 26.00  | 16.00          | 28.00           | 15.60          | 7               |
|              | K7          | 13.00     | 10.50         | 0.00            | 24.00  | 18.00          | 26.00           | 14.00          | 6               |
| Goat milk    | G1          | 11.80     | 8.50          | 19.00           | 18.00  | 10.00          | 15.00           | 9.00           | 7               |
|              | G2          | 8.60      | 8.00          | 8.00            | 16.00  | 9.00           | 17.00           | 7.00           | 7               |
|              | G3          | 13.60     | 9.60          | 13.10           | 21.00  | 16.00          | 21.00           | 13.50          | 7               |
|              | G4          | 13.80     | 9.60          | 11.60           | 24.00  | 10.00          | 17.00           | 11.00          | 7               |
|              | G5          | 0.00      | 0.00          | 0.00            | 15.00  | 0.00           | 14.00           | 0.00           | 2               |
|              | G6          | 0.00      | 0.00          | 0.00            | 0.00   | 0.00           | 13.00           | 0.00           | 1               |
|              | G7          | 0.00      | 0.00          | 10.00           | 20.00  | 0.00           | 15.00           | 0.00           | 3               |
|              | G8          | 13.00     | 11.00         | 18.00           | 23.00  | 13.60          | 26.00           | 15.00          | 7               |
|              | G9          | 13.80     | 14.60         | 14.80           | 25.00  | 12.80          | 24.00           | 11.00          | 7               |
| Cow milk     | C1          | 18.60     | 12.00         | 15.60           | 30.00  | 15.00          | 28.00           | 15.60          | 7               |
|              | C2          | 16.60     | 11.00         | 14.60           | 18.00  | 16.00          | 21.00           | 13.00          | 7               |
|              | C3          | 13.60     | 11.00         | 18.50           | 13.00  | 17.00          | 18.00           | 11.50          | 7               |
|              | C4          | 12.00     | 9.00          | 16.30           | 20.00  | 11.00          | 15.00           | 14.00          | 7               |
|              | C5          | 13.00     | 11.00         | 17.60           | 20.00  | 10.00          | 26.00           | 13.60          | 7               |
|              | C6          | 12.60     | 10.00         | 13.30           | 19.00  | 11.60          | 22.00           | 16.00          | 7               |
|              | C7          | 17.80     | 14.80         | 19.50           | 23.00  | 13.50          | 21.00           | 14.00          | 7               |
|              | C8          | 12.00     | 13.00         | 13.60           | 19.00  | 12.00          | 17.00           | 9.00           | 7               |
|              | C9          | 17.00     | 11.00         | 21.60           | 22.00  | 10.60          | 20.00           | 19.00          | 7               |
|              | C10         | 9.00      | 8.60          | 11.60           | 15.00  | 0.00           | 16.00           | 7.60           | 6               |
|              | C11         | 10.00     | 9.00          | 13.60           | 17.00  | 12.00          | 17.00           | 10.00          | 7               |
|              | C12         | 10.00     | 9.00          | 16.00           | 25.00  | 9.00           | 22.00           | 14.00          | 7               |
|              | C13         | 8.60      | 9.00          | 13.00           | 20.00  | 0.00           | 14.00           | 11.00          | 6               |
|              | C14         | 8.00      | 10.00         | 15.00           | 14.00  | 14.00          | 17.00           | 8.00           | 7               |
|              | C15         | 16.00     | 14.60         | 16.50           | 28.00  | 11.50          | 24.00           | 14.00          | 7               |
|              | C16         | 10.00     | 11.00         | 15.60           | 29.00  | 12.00          | 26.00           | 16.00          | 7               |

\(L. \) helveticus | 10.00 | 13.00 | 15.00 | 14.00 | 14.00 | 17.00 | 10.00 | 7

0.00 = No effect, <7 = Low strong (Resistance), 7-10 mm = strong (Intermediated Resistance), >10 mm inhibition zone = Very strong (Very Sensitivity)
Fig. 1. Disk diffusion plate showing degree and type the antibacterial activity of *Lactobacillus* isolates using inhibition zone (mm).

Table 3. Residual activity (%) of *Lactobacillus* spp. isolates after pasteurization (72°C/15 min) antagonized seven pathogens

| Isolate No. | Residual activity (%) | Pathogenic organisms |
|-------------|------------------------|---------------------|
|             | B. cereus | Staph. aureus | L. monocytogenes | E. coli | Ps. aeruginosa | Shigella sonnei | S. typhimurium |
| K3          | 98.43    | 0.00         | 89.20          | 50.00   | 43.75        | 43.63          | 80.71          |
| K4          | 0.00     | 0.00         | 85.71          | 56.52   | 0.00         | 0.00           | 0.00           |
| K5          | 95.33    | 84.62        | 93.90          | 40.00   | 57.93        | 34.44          | 57.89          |
| K6          | 95.88    | 79.36        | 98.50          | 70.40   | 68.75        | 34.64          | 67.95          |
| G1          | 0.00     | 0.00         | 78.94          | 55.55   | 0.00         | 0.00           | 0.00           |
| G2          | 0.00     | 0.00         | 75.00          | 0.00    | 0.00         | 0.00           | 0.00           |
| G3          | 0.00     | 72.92        | 96.20          | 74.30   | 0.00         | 0.00           | 0.00           |
| G4          | 76.81    | 0.00         | 97.41          | 25.00   | 87.00        | 0.00           | 0.00           |
| G8          | 0.00     | 0.00         | 0.00           | 53.50   | 71.32        | 0.00           | 0.00           |
| G9          | 84.05    | 75.34        | 96.60          | 57.20   | 82.81        | 47.08          | 100.00         |
| C1          | 60.75    | 0.00         | 96.20          | 53.30   | 0.00         | 36.79          | 62.18          |
| C2          | 74.09    | 96.36        | 75.30          | 35.00   | 0.00         | 44.29          | 76.92          |
| C3          | 88.23    | 50.90        | 81.00          | 100.00  | 52.94        | 55.56          | 98.26          |
| C4          | 0.00     | 0.00         | 58.90          | 48.00   | 96.36        | 76.84          | 78.60          |
| C5          | 84.61    | 0.00         | 73.90          | 48.00   | 0.00         | 40.77          | 68.38          |
| C6          | 97.62    | 0.00         | 100.00         | 97.90   | 62.93        | 61.81          | 62.50          |
| C7          | 89.89    | 87.80        | 90.30          | 72.20   | 92.60        | 91.90          | 97.90          |
| C8          | 75.00    | 0.00         | 100.00         | 48.94   | 52.50        | 0.00           | 0.00           |
| C9          | 38.82    | 0.00         | 76.90          | 51.40   | 52.83        | 41.5           | 48.95          |
| C11         | 0.00     | 0.00         | 95.60          | 82.35   | 0.00         | 0.00           | 0.00           |
| C12         | 90.00    | 0.00         | 53.20          | 41.20   | 0.00         | 25.90          | 0.00           |
| C14         | 0.00     | 0.00         | 84.00          | 59.30   | 0.00         | 49.00          | 0.00           |
| C15         | 56.25    | 0.00         | 97.00          | 60.70   | 0.00         | 22.08          | 0.00           |
| C16         | 83.30    | 0.00         | 66.00          | 66.60   | 69.42        | 34.62          | 56.25          |

*L. helveticus* 0.00 0.00 84.00 59.30 0.00 49.00 0.00
Evaluation of Lactobacilli properties and their antibacterial substances by using sweet whey as growth medium against pathogenic bacteria

Fig. 2. 1% Agarose gel electrophoresis showing (Lb) Lactobacillus spp. / DNA - PCR product amplified from total DNA extracted from Lactobacillus spp. using sense and antisense primers. (L) DNA ladder high molecular weight.

(Bf) >200217-029_M04_A4-group10_BF-group10.ab1

TGCCCCAAGCTACTGCTACTACATGCAAGTGCAGCTCCTCACGGAAATGACGTCGTTGACATTG
TTCAACAATGAAGGAGCGAGATGGGAAATCGCCAGAAGAGGGGAAAACGAGGCAACATGGATACGT
TTACGGTGTTTGGTGGTTAAAGAAGAACACCTTTGAGAGTA

Fig. 3. Lactobacillus brevis ATCC 14869 = DSM 20054 16S ribosomal RNA, partial sequence
Table 4. Accession no of Lactobacillus spp. isolates recorded in gene bank was aligned with selected Lactobacillus isolate

| Description                                             | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession   |
|---------------------------------------------------------|-----------|-------------|-------------|---------|------------|-------------|
| Lactobacillus brevis ATCC 14869 = DSM 20954 105 ribosomal RNA, partial sequence | 1559      | 1559        | 100%        | 0       | 98.43%     | NR_116238_1 |
| Lactobacillus brevis strain ATCC 14869 105 ribosomal RNA, partial sequence | 1543      | 1543        | 100%        | 0       | 97.90%     | NR_116474_2 |
| Lactobacillus vororosii strain TLM-87 195 ribosomal RNA, partial sequence | 1487      | 1487        | 100%        | 0       | 96.99%     | NR_104652_1 |
| Lactobacillus cerveus strain TLM BP 10423200-2220 195 ribosomal RNA, partial sequence | 1467      | 1467        | 100%        | 0       | 96.53%     | NR_105030_1 |
| Lactobacillus koreensis DSM 18448 strain CNC150 195 ribosomal RNA, partial sequence | 1465      | 1465        | 100%        | 0       | 96.53%     | NR_116934_1 |
| Lactobacillus paracasei strain LMK 11884 105 ribosomal RNA, partial sequence | 1450      | 1450        | 100%        | 0       | 96.19%     | NR_104246_1 |
| Lactobacillus harveyi strain LMG 11321 195 ribosomal RNA, partial sequence | 1443      | 1443        | 100%        | 0       | 96.00%     | NR_104246_1 |
| Lactobacillus orisporus DSM 21775 = NBRC 105953 195 ribosomal RNA, partial sequence | 1437      | 1437        | 100%        | 0       | 95.96%     | NR_114271_1 |
| Lactobacillus orisporus DSM 21775 = NBRC 105953 strain L11 195 ribosomal RNA, partial sequence | 1425      | 1426        | 100%        | 0       | 95.74%     | NR_114271_1 |
| Lactobacillus psychrophilic strain THU 1713 105 ribosomal RNA, partial sequence | 1417      | 1417        | 99%         | 0       | 95.73%     | NR_110579_1 |
| Lactobacillus pyocyaneus strain P-15015 105 ribosomal RNA, partial sequence | 1373      | 1373        | 99%         | 0       | 94.63%     | NR_104246_1 |
| Lactobacillus acidophilus strain R-10665 105 ribosomal RNA, partial sequence | 1371      | 1371        | 99%         | 0       | 94.71%     | NR_104246_1 |
| Lactobacillus casei strain R-27895 105 ribosomal RNA, partial sequence | 1339      | 1339        | 99%         | 0       | 94.15%     | NR_104251_1 |
| Lactobacillus acidinitrocresolis strain TMV 1.442 105 ribosomal RNA, partial sequence | 1334      | 1334        | 99%         | 0       | 93.96%     | NR_116934_1 |
| Lactobacillus odoratus DSM 19099 = JCM 1541 strain YTT 11844 195 ribosomal RNA, partial sequence | 1330      | 1330        | 99%         | 0       | 93.93%     | NR_112781_1 |
| Lactobacillus helveticus strain YTT10 105 ribosomal RNA, partial sequence | 1308      | 1308        | 99%         | 0       | 93.40%     | NR_110871_1 |
| Lactobacillus kimchii JCM 10003 strain DYC121 105 ribosomal RNA, partial sequence | 1308      | 1308        | 99%         | 0       | 93.49%     | NR_115411_1 |
| Lactobacillus rhamnosus strain R-1762 105 ribosomal RNA, partial sequence | 1296      | 1296        | 99%         | 0       | 93.39%     | NR_115581_1 |
| Lactobacillus paracasei strain JCM 11698 105 ribosomal RNA, partial sequence | 1301      | 1301        | 99%         | 0       | 93.36%     | NR_112781_1 |

Fig. 4. A phylogenetic tree of Lactobacillus isolate revealed 98.43 % a moderate degree of similarity to Lactobacillus brevis ATCC 14869 isolate (Accession no. NR_116238_1)
An antibacterial substratum analysis in fermentation broth \textit{Lactobacillus brevis} (C7)

It was a genus of nonspore, Gram-positive bacteria whose most common feature is the fermentation of sugars into organic acids. The decrease in pH can seriously inhibit other bacteria’s production. In addition, several other studies have found that H$_2$O$_2$ produced during the metabolic process can inhibit bacteria (Charlier et al 2009 and Hu et al 2019). On the other hand, some lactic acid bacteria can produce pathogen-inhibiting bacteriocins and bacteriocin-like compounds (Zhao et al 2016). They can produce a variety of organic acids, mainly Probiotic acid, lactic acid, Citric acid, formic acid, sorbic acid and butyric acid. 15.23, 12.365, 22, 4.96, 3.12, and 1.25 mg/mL respectively in the fermentation broth of \textit{Lactobacillus brevis} (C7) (Table 5 and Fig. 5). The organic acids that have an antimicrobial behavior are the acetic acids and lactic acids in the metabolite products of \textit{Lactobacillus brevis} (C7) (Zalán et al 2010). Additionally, other common organic acids like citric acid may also possess antibacterial activity. The lactic acid, citric acid, was chosen for determination in our study. According to \textit{Lactobacillus brevis} (C7) pH curve, the pH of the fermentation broth stabilizes after 24 hours at 3.80 (Hu et al 2019), five types of organic acids were found in the three strains fermentation broth by HPLC analysis. Organic acid extracted from L. Plantarum strains (P1, S11, and M7) were mainly lactic acid and were the lowest in L. S11 (26.4 g/L) plantarum Against L. Plantarum L. and S11. M7, L. plantarum, the highest concentrations of acetic (3.3 g/L) and lactic acid (2.6 g/L) were detected in plantarum P1. Beyond that, all fermentation broths also detected a tiny bit of tartaric and malic acid. The lactic acid content is the highest compared with other organic acids, and proves to be the key ingredient behind the fall in the pH level of the broth. This reduction has an impact that is inhibitory in nature and directly affects the pathogenic bacteria, effectively stunting its growth.

Optimization of growth of selected strain

Screening of the most significant cell dry weight parameters by using Taguchi design

A total of 5 factors including (NaCl concentration, pH, inoculum size, temperature and incubation period) with four rates were chosen to conduct this optimization phase (which will provide an appropriate error estimate) studied in 16 (n+1) \textit{Lactobacillus} strain C7 experiments. Data in (Table 6), showed that the biomass production was ranged from 0.87 to 0.98 g/l due to the influence of interactions between variables. Maximum cell dry weight (0.98 g/l) was achieved at run number 15, followed by run 11 (0.97 g/l of cell dry weight). The run 15 was the best run which was the optimum conditions for cell dry weight represented 4 g NaCl/100ml, adjusted pH to 7.5 and inoculated with 5% of the \textit{Lactobacillus brevis} (C7) then incubated at 25°C for 72 hours. Results also indicated that the lowest cell dry weight was observed in run number 3 being 0.87 g/l.

Analysis of variance (ANOVA) through the Fisher test has been deployed to examine the impact of variables that are independent when it comes to the response. Substantial results were outlined where the p-value stood at <0.05. Adjusted R2 and multiple determination coefficient (R) were put to use to check on quality so that the fitness of the second order polynomial equation could be evaluated. Adeq Precision “measures the signal to noise ratio, a ratio greater than 4 is desirable, so the ratio of 14.60 indicates an adequate signal and this model can be used to navigate the design space.

The F-value of 18.37 implies that the model is significant. There is only a 1.75% chance that an F-value this large could occur due to noise. The smaller p-value indicates the high significance of the corresponding coefficient.

The analyzed results of biomass production by \textit{Lactobacillus} strain (C7) suggested that out of 5 different independent variables, only 3 variables (NaCl, pH and inoculum size) were significantly affected the cell dry weight. The p-value of these significant variables ranged 0.007 to 0.028 Table 7.

Interpretation of the data was based on the signs (positive or negative effect on the response) and statistical significance of coefficients (P<0.05). Interactions between two factors could appear as an antagonistic effect (negative coefficient).

The coefficient of determination ($R^2$) was 0.99 (which means that 99% of the total variation was explained by the model) by Lactobacillus strain (C7). This indicates a satisfactory representation of the process models and a high determination between the experimental and predicated values for the selected bacteria. By using Design-Expert, the equation obtained for Taguchi design (first order model) of the selected bacteria was as follows:

\[
Y_{\text{cell dry weight}} = +0.9206 +0.0194 \text{(NaCl)} + 0.0069 \text{(pH)} + 0.0194 \text{(inoculum size)} + 0.0069 \text{(temperature)} + 0.0044 \text{(incubation period)}
\]

Where: Y is the predicated response.
Table 5. Organic acids in fermentation broth of *Lactobacillus brevis* C7 by HPLC.

| RF  | Compound       | Concentration mg/mL |
|-----|----------------|---------------------|
| 9.3 | Sorbic acid    | 3.12                |
| 10.02 | Formic acid | 4.96                |
| 10.32 | Citric acid   | 5.22                |
| 15.0 | Probionic acid | 15.23              |
| 18.12 | Butyric acid  | 1.25                |
| 18.61 | Lactic acid   | 12.36               |

Fig. 5. HPLC Chromatograph chart showing organic acids in fermentation broth of *Lactobacillus* strain C7 (*Lactobacillus brevis*). Data are the mean ± SD of at least three independent experiments.

Table 6. Taguchi experimental design matrix and cell dry weight real values of the *Lactobacillus brevis* (C7).

| Run no. | A | B | C | D | E | Cell dry weight (g/L) |
|---------|---|---|---|---|---|-----------------------|
| 1       | 6.5 | 7   | 5  | 40 | 24 | 0.88                  |
| 2       | 4  | 5.5 | 7  | 40 | 48 | 0.92                  |
| 3       | 2  | 7   | 10 | 25 | 48 | 0.87                  |
| 4       | 4  | 6.5 | 10 | 35 | 24 | 0.91                  |
| 5       | 0  | 7   | 7  | 35 | 72 | 0.88                  |
| 6       | 2  | 6.5 | 2  | 40 | 72 | 0.93                  |
| 7       | 6.5 | 5.5 | 10 | 30 | 72 | 0.90                  |
| 8       | 2  | 5.5 | 5  | 35 | 96 | 0.93                  |
| 9       | 2  | 7.5 | 7  | 30 | 24 | 0.91                  |
| 10      | 4  | 7   | 2  | 30 | 96 | 0.90                  |
| 11      | 0  | 6.5 | 5  | 30 | 48 | 0.97                  |
| 12      | 0  | 5.5 | 2  | 25 | 24 | 0.96                  |
| 13      | 6.5 | 6.5 | 7  | 25 | 96 | 0.90                  |
| 14      | 0  | 7.5 | 10 | 40 | 96 | 0.95                  |
| 15      | 4  | 7.5 | 5  | 25 | 72 | 0.98                  |
| 16      | 6.5 | 7.5 | 2  | 35 | 48 | 0.94                  |

A=Nacl   B=pH    C=Inoculum size    D=Temperature  E=Incubate period
Table 7. Statistical analysis of variance (ANOVA) of Taguchi design for cell dry weight by the *Lactobacillus brevis* strain (C7).

| Source          | df | Mean Square | F-value | p-value |
|-----------------|----|-------------|---------|---------|
| Model           | 15 | 0.0013      | 18.37   | 0.018*  |
| A-Nacl          | 3  | 0.001       | 14.26   | 0.028*  |
| B-pH            | 3  | 0.0029      | 39.17   | 0.007*  |
| C-Inoculum size | 3  | 0.0014      | 18.6    | 0.019*  |
| D-Temperature   | 3  | 0.0001      | 1.46    | 0.382   |
| E-Incubation period | 3 | 0.0001     | 0.0255  | 0.993   |
| Std. Dev.       |    | 0.0085      |         |         |
| Mean            |    | 0.9206      |         |         |
| C.V. %          |    | 0.9275      |         |         |

The Predicted $R^2$ of 0.62 is not as close to the Adjusted $R^2$ of 0.93 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.6 indicates an adequate signal. This model can be used to navigate the design space. Data illustrated by Fig. (6) indicated the actual values related with the predicted values in the same line.

Therefore, the present $R^2$ values hinted that the model is reliable for biomass production in the present study.

So, the significant factors (NaCl, pH and Inoculum size) will be optimized using Central composite design in the next study.

Second design the Central composite design (CCD) and response surface methodology (RSM)

After selecting the most significant variables influencing cell dry weight by the *Lactobacillus* strain C7. A Central composite design (CCD) was performed to determine the optimal levels of the selected significant variables and optimum amount of cell dry weight. Results of 20 experiments with different combination of salinity ratio (A), pH (B) and inoculum size (C) represented in (Table 8).

The *Lactobacillus* strain (C7) was observed that the maximum cell dry weight value (0.98 g/l) was achieved at runs number (1,4,5,6,13 and 19) in the presence of NaCl (5.25 g/100ml) at PH(7.75) with inoculum size (7.5%) , were attributable to the lowest cell dry weight that was observed in run number 9.

The best runs which represent the same result of actual and predicted cell dry weight, whereas the runs (1,4,5,6,13 and 19) being 0.98 g/l for actual and predicted, respectively (Fig. 7).

The statistical significance of the model was checked by F-test and ANOVA for the response surface quadratic model (summarized in (Table 8) by the *Lactobacillus* strain C7. The model F-value of 2.39 implies there is a 9.56% chance that a F-value this large could occur.

The $p$-value less than 0.0500 indicate model terms are significant. In this case $A^2$, $C^2$ are significant model terms. The values are greater than 0.1000 indicate the model terms are not significant. The determination coefficient $R^2$ of the model was 0.68 for the *Lactobacillus* isolate indicated that the model explained 68% of the total variations and revealed excellent agreement between the experimental results and the predicted values calculated from the model. $R^2= \pm 1$, a straight-line relationship exists between the experimental and predicted cell dry weight.

$^*$Significant at 5% level ($p<0.05$), df=degree of freedom, $p=corresponding level of significance, F=corresponding level of significance, Std. Dev = Standard Deviation, C.V.= coefficient of variation and $R^2$=Determination coefficient. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 4.160 indicates an adequate signal. This model can be used to navigate the design space. Therefore, the present $R^2$ values hinted that the model is reliable for biomass production in the present study.
(C.V.) indicates a better precision and higher reliability of the experiment. This indicates that C.V. = 3.00% for the *Lactobacillus* strain (C7), of this model is perfect. The p-value, less than 0.05, indicated that lack of fit for the model is significant. The mathematical model of the *Lactobacillus* strain (C7) describing the relationship between variables (A, B and C) and response (Y) for cell dry weight could be obtained by the following second order polynomial equation:

\[
Y_{\text{cell dry weight}} = +0.9813 +0.0012A +0.0161B +0.0044C -0.0025(AB) +0.0025(AC) +0.0100(BC) -0.0208(A^2) -0.0155(B^2) -0.0190(C^2)
\]

Where: Y is the predicted response.

Three-dimensional response surface and two-dimensional contour plots are graphical based on the model equation. These plots used to explain the interaction among variables and determine the optimum level of each factor on cell dry weight. Each figure presents the effect of 3 factors while the other factor was held at optimum level. For 2D and 3D graphs, revealed that the F-value is defined as the ratio of the mean square due to regression to the mean square due to error. The probability value of (A^2) and (C^2) (p-value <0.05) is the most highly significant variable compared to linear, interaction and other quadratic variables.

**Fig. 6.** The actual and predicted values of Taguchi design of the *Lactobacillus brevis* strain (C7).

**Fig. 7.** The actual and predicted values of (CCD) design of the *lactobacillus brevis* strain (C7).
Evaluation of Lactobacilli properties and their antibacterial substances by using sweet whey as growth medium against pathogenic bacteria

Table 8. Central composite design matrix of independent variables used in cell dry weight (actual, predicted values) for the Lactobacillus brevis strain (C7).

| Run no. | Variables | Cell dry weight g/l |
|---------|-----------|---------------------|
|         | A   | B     | C     |               |
| 1       | 5.25| 7.7   | 7.5   | 0.98          |
| 2       | 6.5 | 7.5   | 5     | 0.88          |
| 3       | 5.25| 7.7   | 12    | 0.95          |
| 4       | 5.25| 7.7   | 7.5   | 0.98          |
| 5       | 5.25| 7.7   | 7.5   | 0.98          |
| 6       | 5.25| 7.7   | 7.5   | 0.98          |
| 7       | 3   | 7.7   | 7.5   | 0.94          |
| 8       | 5.25| 7.7   | 3     | 0.95          |
| 9       | 4   | 7.5   | 10    | 0.87          |
| 10      | 6.5 | 8     | 5     | 0.92          |
| 11      | 4   | 8     | 5     | 0.92          |
| 12      | 4   | 8     | 10    | 0.96          |
| 13      | 5.25| 7.7   | 7.5   | 0.98          |
| 14      | 5.25| 8.2   | 7.5   | 0.96          |
| 15      | 4   | 7.5   | 5     | 0.89          |
| 16      | 6.5 | 7.5   | 10    | 0.89          |
| 17      | 6.5 | 8     | 10    | 0.95          |
| 18      | 5.25| 7    | 7.5   | 0.96          |
| 19      | 5.25| 7.7   | 7.5   | 0.98          |
| 20      | 7   | 7.7   | 7.5   | 0.95          |

| Variable | Symbol | Real levels |
|----------|--------|-------------|
| NaCl     | A      | 4           6.5 |
| pH       | B      | 7.5         8   |
| Inoculum size | C | 5           10 |

-1=low level of the variable and +1=high level of the variable

Table 9. Statistical analysis of variance (ANOVA) of (CCD) design for cell dry weight by the Lactobacillus strain (C7).

| Source                  | df | Mean Square | F-value | p-value |
|-------------------------|----|-------------|---------|---------|
| Model                   | 9  | 0.0019      | 2.39    | 0.0956  |
| A-A Nacl                | 1  | 0.0000      | 0.0258  | 0.8755  |
| B-B pH                  | 1  | 0.0035      | 4.42    | 0.0618  |
| C-C Inoculum size       | 1  | 0.0003      | 0.3288  | 0.5791  |
| AB                      | 1  | 0.0000      | 0.0624  | 0.8079  |
| AC                      | 1  | 0.0000      | 0.0624  | 0.8079  |
| BC                      | 1  | 0.0008      | 0.9977  | 0.3414  |
| A²                      | 1  | 0.0062      | 7.78    | 0.0191* |
| B²                      | 1  | 0.0035      | 4.32    | 0.0644  |
| C²                      | 1  | 0.0052      | 6.51    | 0.0288* |
| Residual                | 10 | 0.0008      |         |         |
| Lack of Fit             | 5  | 0.0016      |         |         |
| Pure Error              | 5  | 0.0000      |         |         |

| Cor Total               | 19 |             |         |         |

| Std. Dev. | Mean  | C.V. % | R²    | Adjusted R² | Adeq Precision |
|-----------|-------|--------|-------|-------------|----------------|
| 0.0283    | 0.9435| 3.00   | 0.6825| 0.3968      | 4.1597         |

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Fig. 8. 3-dimensional response surface showing the effect of NaCl, pH and Inoculum size on biomass production by the *Lactobacillus brevis* strain (C7).

**a)** NaCl vs. pH

**b)** NaCl vs. inoculum size

**c)** pH vs. inoculum size
Similar response data were presented in (Fig. 8). This plot indicates that the optimum conditions for the highest cell dry weight under in media contained 5.25 g Nacl/100ml and adjusted pH to 7.7 then inoculated with 7.5% of standard inoculum size and incubated at 25°C for 72 hours. Data in (Figs. 8 a-c) indicated that the interaction between Nacl concentrations vs. pH at 7.5% of inoculum size (Fig. 8a). The interaction between Nacl concentrations vs. inoculum size at pH 7.7 (Fig. 8b). In (Fig. 8c) showed the interaction between pH and inoculum size at 5.25 g Nacl/100ml. From the previous results, it could be summarized that the production of biomass using two designs Taguchi and CCD for optimized production were not difference in cell dry weight being 0.98 g/l.

CONCLUSION

In the present study, 32 LAB isolates were isolated from raw milk and Cottage cheese using the specific De Man, Regosa-Sharp medium and were identified as members of the genus Lactobacillus spp. Sweet whey low-cost by-products proved to be suitable medium for growth and production of antibacterial substances by these isolates. Cell free supernatants (CFS) of these isolates showed potential to antagonize the seven foodborne organisms and pathogens including Gram-positive and Gram-negative bacteria.

These active isolates were good acid producers causing a drop in pH of supernatants between 3.7 and 4.9, but activities completely disappeared by neutralization. In addition inhibition due to hydrogen peroxide was excluded because no sensitivity to catalase was detected. Therefore, antibacterial activity could be attributed mainly to organic acids production and to certain extent to bacteriocin compounds which were inactivated by neutralization. CFS of five of these isolates withstanded pasteurization treatment. C7 isolate showed the highest relatively residual activities and was selected, and identified as Lactobacillus brevis. This strain Lactobacillus brevis considered a hetero fermentative to produce co2 and many organic acids including propionic acid, lactic acid. Citric acid, Formic acid, Sorbic acid and Butyric acid with 15.23, 12.36 5.22, 4.96, 3.12 and 1.25 mg/mL. High mass production of this strain could be achieved by growing at pH 7.75 and 25°C at salt concentration of 5.25% NaCl and inoculum size 7.5% with incubation period 72 hours.

Therefore, the growth of Lactobacillus on the sweet whey low-cost by-product will certainly reduce production on the industrial scale. The aforementioned characteristics of this strain together with its strong antibacterial activity against of Gram-positive and Gram-negative pathogens recommended its use as bio-preservative for fermented dairy products which are subjected to harsh heat treatment and could be functional for the production of some cheese which contain relatively high salt concentration.

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تقييم خصائص العصيات اللبنية (Lactobacilli) ومودها المضادة للبكتيريا باستخدام الشرش الحلو كبيئة للنمو ضد البكتيريا المسببة للأمراض

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خالد عبدالفتاح الدجدج۱، خديجة أحمد أبوطالب۱، شيماء عبدالرءوف أمين۱
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۲- المركز الأكاديمي للأغذية والأعلاف – مركز البحوث – الدفي – مصر

الموجز

أدى الطلب المتزايد من المستهلكين الذين يبحثون عن منتجات آمنة طبيعية والمخاطر الصحية المحتملة للمنتجات الغذائية المحفوظة كيميائياً والمعالجة كيميائياً إلى ظهور تقنيات بديلة للحفاظ على جودة المنتجات الغذائية والحفاظ عليها. واحدة من تقنيات الحفظ هذه هي استخدام بكتيريا حمض اللاكتيك كبابدئات للحفاظ عل

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