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ISOLATION, CHARACTERIZATION AND DETERMINATION OF PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA FROM EFFLUENTS OF A DAIRY PLANT IN MAURITIUS

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ABSTRACT

Probiotics are live microorganisms that have beneficial effects on their host's health. The objective of this study was to isolate lactic acid bacteria (LAB) from dairy effluents and to evaluate the probiotic potential of the isolated microorganism(s). Briefly, LAB was isolated from dairy effluents and subjected to microscopic examination and a panel of assays to evaluate their acid and bile tolerances, growth characteristics, carbohydrate fermentation pattern, antimicrobial activity and their antibiotic resistance pattern. The identity of the isolate was confirmed by amplification of 16S ribosomal DNA (rDNA) and 16S rDNA sequencing. The isolate was identified as a gram-positive catalase-negative heterofermentative coccobacillus able to metabolize maltose, lactose and sucrose only. It tolerated up to 4% NaCl concentrations and only grew at 37°C. The isolate exhibited resistance to stomach pH (pH = 3.0) and tolerance against 0.3% bile. The isolate also displayed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* and was resistant to ampicillin, erythromycin, streptomycin and tetracycline. Molecular identification revealed the organism to be Leuconostoc mesenteroides. Taken together, this study shows that dairy effluent is a source of probiotic LAB and points to its potential as a valuable by-product of milk processing.

Keywords: Probiotics, Dairy, Effluent, Lactic Acid Bacteria, Leuconostoc

INTRODUCTION

Today's consumers consider food not only in terms of taste and immediate nutritional needs, but also in terms of their ability to provide specific health benefits beyond their basic nutritional value **[1]**. In recent years, lactic acid bacteria (LAB), which are generally recognized as safe (GRAS) organisms used commercially in dairy products including yoghurt, kimchi, and other fermented foods, have become well known as probiotics. LAB generate lactic acid from sugars (including lactose) and other carbohydrates, thus contributing to acidification, flavouring and other characteristics associated with a host of dairy products **[2]**.

Etymologically, the word probiotic is derived from the Greek language 'pro bios', which means 'for life' as opposed to 'antibiotics' which means 'against life' **[3]**. As per the World Health Organization and the Food and Agriculture Organization of the United States, probiotics are defined as "live microorganisms which when administered in adequate amounts, have a beneficial effect on health of the host organism" **[4]**. Functional food is described as food containing ingredients with positive effects on host health beyond their nutritive value and those products contain biologically active components that improve health, such as probiotics. Currently, the largest segment of the functional food market is dominated by healthy products targeting the balance and activity of the intestinal microflora **[1]**. The probiotic market is thus expanding worldwide, including Europe, some parts of Asia, and the U.S. Europe represents the largest as well as the fastest-growing market in probiotics followed by Japan, the second largest market which account for about 45% of the probiotic market **[5]**. In the quest for new strains of probiotic LAB, numerous researchers have isolated probiotic microorganisms from traditionally fermented foods **[6]**, although dairy effluents are equally rich in probiotics **[7]**. In fact, dairy effluents have a preponderant flora of lactic acid bacteria **[8]**. Dairy wastes are produced in large volumes by the dairy industry, both solids and liquids, and this poses an escalating disposal and pollution problem. Hence, the wastes can be exploited as inexpensive sources of new probiotic strains of LAB to produce value-added products.

Faced with the need to continuously search for novel probiotic strains, the objective of this study was to isolate, characterize and evaluate the probiotic potential of LAB sourced from effluents of a dairy industry from Mauritius.

MATERIALS AND METHODS

2.1. Sample collection

Dairy effluent samples were obtained from Maurilait Production Ltd (Phoenix, Mauritius). They were aseptically collected in sterile containers and stored on ice until delivery to the laboratory. In the laboratory, samples were stored in the refrigerator at 4ºC and analyzed within 24 h of collection.

2.2. Isolation of Lactic Acid Bacteria from dairy effluents

Effluent samples were serially diluted in 0.1% sterile peptone water. An aliquot of 0.1ml of the samples and their ten-fold serial dilutions were spread-plated on regular MRS (rMRS) and modified MRS (mMRS) supplemented with sodium azide (NaN_3), sucrose and bromocresol purple **[9]**, at a concentration of 0.2g/l, 20g/l and 0.12g/l, respectively. The plates were incubated at 37°C for 120 hours. After incubation, individual colonies were selected and streaked onto mMRS agar plates at 37°C and their colonial morphology examined. The strains were subcultured twice in MRS broth and incubated overnight at 37°C, prior to use **[10]**. Isolates were then subjected to biochemical confirmation tests.

2.3 Confirmation tests of LAB isolate

2.3.1 Gram staining and catalase test

Gram staining was performed on a fresh culture of the isolate grown in MRS broth at 37°C for 24 h. Catalase test was performed by streaking a loopful of colony material of the isolate growing on mMRS agar onto a glass slide. A drop of 3% hydrogen peroxide (H_2O_2) solution was placed on the cells and the slide covered with a petri plate in order to contain any aerosols formed during the reaction. The absence of evolution of oxygen gas revealed the microorganism to be catalase-negative and therefore a potential probiotic.

2.3.2 Carbohydrate fermentation characteristics

In order to determine the homofermentative or heterofermentative characteristics of the isolate, production of CO_2 from glucose was tested by a protocol adapted from Vumie **[11]**.

Briefly, 50 µl of overnight 50 fresh cultures were incubated in a phenol red glucose broth with Durham tube at 37°C for 5 days. Evidence of gas (CO_2) and acid production was noted as visible air bubbles trapped inside the Durham tubes and a colour change from red to yellow respectively.

The isolate was also characterized based on its ability to ferment eight different carbohydrates (arabinose, lactose, maltose, sucrose, ribose, rhamnose, mannitol and sorbitol) according to a protocol adapted from Reiner **[12]**. Fifty µl of overnight cultures was inoculated into nutrient broth containing phenol red indicator supplemented with the carbohydrate of

interest and incubated at 37ºC for 5 days. After incubation, the colour change from red to yellow was recorded as a positive fermentation result when compared with the negative control, which remained red after incubation.

2.4 Evaluation of probiotic properties

2.4.1 Growth at different temperatures and NaCl concentrations

This experiment was done according to Yavuzdurmaz **[3]**. Briefly, 5 ml of test media consisting of MRS broth containing bromecresol purple indicator at a concentration of 0.12g/l was prepared and transferred into tubes. Fifty 50 µl of overnight cultures inoculated into tubes were incubated for 7 days at 4, 37 and 45°C. During the incubation period, the culture was qualitatively observed for a colour change from purple to yellow.

Additionally, the isolate was tested for its tolerance to different NaCl concentrations (4% and 6.5% NaCl). Test media containing bromecresol purple indicator at a concentration of 0.12g/l and 4% or 6.5% NaCl, were prepared. Five ml were transferred into tubes which were then inoculated with 50µl of overnight cultures and subsequently incubated at 37°C for 7 days. The change of colour from purple to yellow was indicative of cell growth.

2.4.2 Resistance to low pH and bile salts

Determination of the probiotic properties of the isolate was also done on the basis of its resistance to low pH and tolerance to bile salts. The isolate was grown overnight in 2 ml of MRS broth at 37°C, then sub-cultured into 2 ml of fresh MRS broth and incubated for another 24 h to reach the late exponential phase. The culture was then centrifuged at 8000 rpm for 10 min at 4°C and the cell pellet was re-suspended in 2 ml of MRS broth previously adjusted with HCl to a final pH value of 3. The culture was incubated at 37°C for up to 24 h.

Samples were taken at 0, 1, 2, 3 and 24 h from the onset of incubation to determine the survivability of the cells. Briefly, a 100-µl aliquot of the culture and its 10-fold serial dilutions were plated on MRS agar medium. Plates were incubated at 37°C for 48 h and the LAB counts were expressed in colony forming units per milliliter (cfu/ml). A positive control consisting of regular MRS broth inoculated with the culture was simultaneously set up **[13]**.

The isolate was also grown in 2 ml of MRS broth containing 0.3% and 0.5% of bile salts(Oxgall, USA) and incubated at 37°C for 24 h. Samples were taken at 0, 15, 30, 60, 120, 240 and 1440 minutes from the onset of incubation to determine the survivability of the cells and analyzed as described previously. A positive control consisting of plain MRS broth without bile salts inoculated with the culture was

simultaneously set up.

2.4.3 Antimicrobial activity

The agar disc diffusion method consisting of a sterile paper with a 6 mm diameter **[1]** impregnated with the cell filtrate of the isolate was used to determine the antimicrobial property against two indicator foodborne pathogens, *E. coli* and *S. aureus*. A lawn of the indicator microorganisms was first made by spreading 0.1 ml of the cell suspension of *E. coli* or *S. aureus* over the surface of nutrient agar. The plates were allowed to dry and a sterile paper with a 6mm diameter was placed on the agar. Twenty microlitres of cell-free culture filtrate of the isolate was aliquoted on the centre of the paper and the plate incubated at 37°C for 48h. The diameter of inhibition zones around the paper were then measured.

2.4.4 Antibiotic susceptibility tests

Disc diffusion method was used to test the susceptibility of the isolate against selected antibiotics. The antibiotics in this study were ampicillin (10 µg/ml), erythromycin (15 µg/ml), streptomycin (10 µg/ml) and tetracycline (30 µg/ml) **[2]**. An aliquot of 100 µL of an overnight culture of the isolate was spread onto MRS plates and discs impregnated with antibiotics were positioned on the MRS agar, and then they were incubated for 24 h at 37°C. The sizes of the resulting inhibitory zones were then measured.

2.5. Molecular Identification

2.5.1 Genomic DNA Isolation and PCR Amplification of 16S rDNA

Genomic DNA of the bacterial isolates was extracted using a protocol adapted from Ausubel *et al*. **[4]**. For the amplification of 16S rDNA region of isolate, forward primer 5'- AGTTTGATCATGGCTCAG-3' and reverse primer 5'-TTACCGCGGCTGGCA-3' were used. PCR reaction was carried out in a volume of 25µl containing 1X reaction buffer, 0.2mM dNTP, 20 pmol of the reverse and forward primer, 1 unit of Taq polymerase, 50 ng genomic DNA and made up to a final volume with nanopure water. Lastly, a thermal cycler (Bio-Rad Mycycler) was used for amplification of the DNA. The PCR conditions were as follows: 94°C for 5 min, denaturation for 1 min at 94°C followed by the annealing reaction at 56°C for 1 min then elongation at 72°C for 1 min and finally for 10 min at 72 °C.

2.5.2. Sequencing of the PCR products

PCR amplicons were purified using Fermantas PCR purification kits following the manufacturers' instructions.DNA sequencing reactions were done using a Big Dye Terminator v. 3.1 cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Sequencing reaction products were purified by Exo SAP method. All DNA sequences

were determined with an ABI 3500 DNA sequencer (Applied Biosystems) at Inqaba Biotechnical Industries (Pty) Ltd, South Africa.

2.5.3. DNA sequence Analysis

Forward and reverse sequences were assembled and edited using BioEdit Sequence Alignment Editor version 5.0.9. All sequences were deposited in GenBank. Sequence similarity was estimated by searching the homology in the Genbank DNA database using BLAST. The isolate was then identified based upon the sequence.

3. RESULTS AND DISCUSSION

3.1. Isolation and characterization of Lactic Acid Bacteria from dairy effluents

Many white to cream colonies were readily observed on rMRS agar plates within less than 24h at 37°C while visibly less growth appeared on mMRS agar after extended incubation of 120 hours at the same temperature. The time-to-stationary phase of LAB typically ranges from two to six days, however, growth was observed in less than 24 hours on the rMRS plates suggesting the growth of non-LAB. Colonies from rMRS plates streaked onto mMRS were unable to grow thereby confirming that the bacterium was a non-LAB. An important supplement of mMRS is bromocresol purple, which is a pH indicator that changes colour from purple around neutral pH to yellow below pH 5. It is a differential agent that allows discrimination of LAB from non-LAB [9]. Modified MRS agar also contains sodium azide, a selective agent, since it is a potent iron-porphyrin inhibitor and is effective in inhibiting the growth of most non-LAB and fungi on the mMRS plates. Since LAB do not synthesize iron-porphyrin, their growth is not inhibited in the presence of sodium azide **[9]**.

3.2. Confirmation tests of the LAB isolate

Yellowish orange circular colonies with a convex elevation, entire margin and a large peripheral yellow zone on mMRS were selected for further investigations. Cells of the isolate were found to be Gram-positive catalase-negative coccobacilli occurring in pairs and/or in short chains thus corroborating their identity as a probable LAB. Genera of rod-shaped LAB include *Lactobacillus*, *Bifidobacterium*, *Carnobacterium*, *Bacillus* and *Sporolactobacillus* while common coccus-shaped LAB include *Lactococcus, Streptococcus, Enterococcus* and *Pediococcus*. Morphologically, leuconostocs generally appear as cocci similar in size and shape to lactococci. However, some leuconostocs tend to have a coccoid or coccobacillary morphology due to cell elongation **[14]**. Microscopic examination of the isolate suggests that the microorganism

belongs to the *Leuconostoc* genus. The isolate was also found to be catalase-negative. Catalase is an enzyme produced by many microorganisms that breaks down hydrogen peroxide into water and oxygen and causes gas bubble formation **[3]**. The absence of O_2 gas thus implies the inability of the microorganism to synthesize the iron-porphyrin-containing catalase enzyme which would have otherwise reacted with H_2O_2. Given that *Leuconostoc* cells are catalase-negative coccobacilli often associated with milk and dairy products **[10]**, our findings point to a possible *Leuconostoc* strain.

3.3. Carbohydrate fermentation characteristics of the isolate

With respect to incubation test in phenol red glucose broth, the isolate was found to produce gas at the top of the inverted Durham tube with a colour change from red to yellow. These observations indicate that the isolate is heterofermentative. i.e., is able to ferment glucose to produce D (-) lactic acid, ethanol and CO_2. Most leuconostocs are fermentative and are able to produce CO_2 from glucose. Indeed, Benmechernene *et al*. **[15]** showed that all 83 strains of *L. mesenteroides* isolated from 12 raw camel milk samples were able to produce carbon dioxide from glucose.

With respect to the carbohydrate fermentation patterns of the isolate, it was able to ferment the disaccharides maltose, sucrose and lactose but not arabinose, rhamnose, manitol, sorbitol and ribose. The carbohydrate fermentation profile of the isolate is summarized in Table 1 below.

The term "fermentation" is often used to describe the breaking down or catabolism of a carbohydrate under anaerobic conditions. Bacteria capable of fermenting a carbohydrate are usually referred to as facultative anaerobes [12]. Different bacteria catabolize different energy sources in the medium depending on the specific enzymes synthesized. Most bacteria possess the enzyme systems required for the oxidation and utilization of the simple sugar, glucose. However, not all bacteria have the ability to degrade complex carbohydrates such as lactose and sucrose. The ability to synthesize specific enzymes for utilization of certain carbohydrates provides a distinguishing biochemical tool that aid in the identification of unknown bacteria. The metabolic end-products of fermentation depend on the organisms involved in the fermentation reaction, the substrate being fermented, the enzymes involved, and environmental factors such as pH and temperature. Common end-products of bacterial fermentation include organic acids such as lactic acid, formic acid, acetic acid and butyric acid. Acidification of the media containing pH indicator phenol red yields a

visible colour change from red to yellow. Our results for the carbohydrate fermentation tests are in good agreement with those of Benmechernene *et al*. **[15]**.

3.4. Growth characteristics of the isolate

Another criterion for the evaluation of the probiotic trait of the isolate is the ability to grow at different temperatures. Our findings reveal that the isolate could not grow at 4°C or 45°C over the 7-day incubation period although it was able to grow at 37 °C (data not shown). Hence the isolate can be categorized as a mesophile. According to Benmechernene *et al*. **[15]**, *Leuconostoc* strains isolated from raw camel milk were also classified as mesophiles as they were able to grow at 30 and 37°C but not at 4 and 45°C. Moreover, the halotolerance of the microorganism was also demonstrated when the isolate grew in broth containing 4% NaCl but not 6.5% NaCl (data not shown). Benmechernene *et al*. **[15]** isolated 83 *Leuconostoc* strains from raw milk samples; of which 87% were able to grow in 3% NaCl while only 18% tolerated a higher salinity of 6.5% NaCl. Azadnia and Khan **[16]** also reported that *Leuconostoc mesenteroides* was able to grow at 4% NaCl concentration but not at 6.5% NaCl concentration.

3.5. Resistance to low pH and bile salts

With respect to its acid tolerance, the isolate was found to survive in pH of 3.0 for > 24 h at 37°C. Although there was a decrease in the population by ca. 3.6 log cfu/ml over the first 3 hours of exposure to high acidity, a surviving population of > 5 log cfu/ml was observed. Resistance to low pH is one of the major selection criteria for probiotic strains **[17]**. Before reaching the intestinal tract, probiotic bacteria must first survive the acidity of the stomach **[18]**. Hence, the pH of the *in vitro* assay was adjusted to mimic the gastric pH. Since the bulk luminal pH of the stomach typically ranges from 1 to 3, a pH value of 3.0 was chosen since there is a significant decrease in the viability of strains at pH 2.0 or lower **[3]**. The exposure times to low pH were set for 1, 3, 4 and 24 h since the average residence time of food in the stomach is ca. 3 hours **[3]** but can vary widely from person to person, either naturally or due to several factors. In addition, the time for digestion varies for different types of food **[13]**. The population of the isolate declined rapidly by 3.6 log cfu/ml over the first 3 h. After a cumulative exposure of 24 h, a residual population of 5.5 log cfu/ml was observed as indicated in Figure 1 below.

Benmechernene *et al*. **[15]** demonstrated that strains of *Leuconostoc mesenteroides* subsp. *mesenteroides* isolated form camel milk showed a high survival rate following exposure at low pH (2, 3 and 4). The author noted a decrease in the population by

21.17% at pH 2 but increases of 0.49% and 5.06% at pH 3 and 4 respectively. Similarly, Grosu-Tudor and Zamfir **[13]** observed high survival rates of *Leuconostoc citreum* 344 and *Leuonostoc mesenteroides*. 348 of the order of 10^8 cfu/ml, following a 3 h-exposure at pH 3. After 24 h of incubation at pH 3.0 however, the viability decreased to 10^4 CFU/ml. The usual tolerance of probiotic strains to low pH can be attributed to the physico-chemical characteristics of the source where they are isolated. Probiotic LAB isolated from dairy samples typically ferment milk sugar lactose into lactic acid. During acidification of milk, the pH decreases from 6.7 to 4.6. Therefore, it can be presumed that the pH of the dairy effluents is $\leq$ 4.6 **[19]**. Hence, it is possible that the isolate of this study was the dominating species in the dairy effluents, reflecting a possible adaptation to the specific environment **[17]**. Since probiotic LAB is known to exhibit high tolerance to bile in the human GI tract, the isolate was incubated in the presence of 0.3% bile salts for 4 h in an *in vitro* bile assay to simulate conditions of the human GI tract. Although the bile concentration of the human GI tract varies, the mean intestinal concentration is believed to be 0.3% and the transit time of food in the small intestine is thought to range from 2 to 4 hours **[3]**. After a 2 h-incubation, the viability of the strain declined by 5.3 log cfu/ml and after a cumulative exposure of 4 hours, the population dropped by 6.0 log cfu/ml. However, the population was found to stabilize around 3 log cfu/ml beyond 4 h. Figure 1 below shows the survivor bar chart of the isolate in the presence of bile salts marked by an initial steep decline for the first 4 h followed by tailing thereafter, indicating that the strain eventually started to adapt to the environment.

Indeed, an important characteristic of probiotic bacteria is their ability to survive in the presence of bile salts in the upper part of the small intestine **[20]**. Presence of bile salts belongs to the host of factors that may significantly affect the viability of beneficial LAB in the gastrointestinal (GI) tract, influencing the health of the host. Tolerance to bile salts is thus a prerequisite for colonisation and metabolic activity of bacteria in the small intestine of the host **[13]**. This helps LAB to reach the small intestine and colon, thereby contributing in balancing the intestinal microflora **[13**]. The resistance of probiotic LAB to bile salts might be attributed to its ability to produce bile salt hydrolases **[20]**. Bile salt hydrolases (BSH) protect the producing cells from the toxicity of conjugated bile salts by deconjugating the bile acids **[2]**. Compared with their conjugated counterparts, deconjugated bile acids have decreased

solubility and diminished detergent activity and may, therefore, be less toxic to bacteria in the intestine **[13]**. Grosu-Tudor and Zamfir [13] reported that *Leuconostoc citreum* 344 and *Leuconostoc mesenteroides* 348 were bile tolerant, with survival rates of the order of 10^5 and 10^8 cfu/ml after 24 h of incubation in MRS medium supplemented with 0.3% (w/v) bile salts. Allameh *et al*. **[21]** showed the ability of *L. mesenteroides* subsp. *mesenteroides* to survive and proliferate in the presence of 0.3% bile salts following exposure of 2-8 h. Contrary to the findings reported by these authors, Surono **[22]** found that *L. mesenteroides subsp. mesenteroides* IS-27526 had a poor survival rate of 4.37 log CFU/mL in the presence of 0.3% oxgall (w/v).

3.6. Antibacterial activity of isolate

One of the chief prerequisites of a functional probiotic is its ability to exhibit antagonistic property against foodborne pathogens. The isolate of this study however exhibited relatively poor antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* with a mean inhibition zone diameter of 15 mm and 8 mm respectively (data not shown). Bettache *et al*. **[23]** compared the antagonistic activity of several leuconostocs (*Leuconostoc mesenteroides* subsp. *dextranicum, Leuconostoc citreum* and *Leuconostoc lactis*) against the indicator microorganisms *Staphylococcus aureus, Escherichia coli* and *Listeria innocua*. Contrary to our findings, none of the *Leuconostoc* species could inhibit the test microorganisms **[23]**. On the contrary, Zhang *et al*. **[24]** showed differential inhibitory activity of *Leuconostoc lactis* against test microorganisms *S. aureus* and *E. coli*. The cell-free supernatant of the isolate was most inhibitory against *E. coli* with an mean inhibition zone diameter of 13.7 mm but exhibited low antibacterial activity against *S. aureus* with a mean inhibition zone diameter of 9.5mm **[24]**. Benmechernene *et al*. **[15]** showed that *L. mesenteroides* isolated from Algerian raw milk displayed differential antibacterial activity against *L. ivanovii, L. innocua, S.aureus, E. coli* and *L. plantarum* with diameters of inhibition between 8.8 ± 0.5 mm and 1 mm **[15]**. Taken together, we can infer that the antibacterial activity varies depending on the *Leuconostoc* species assayed **[15]**.

3.7. Antibiotic resistance patterns of isolate

With respect to antibiotic susceptibility of the isolate, it was found to be resistant to all four antibiotics (streptomycin, tetracycline, erythromycin and ampicillin) as no zone of inhibition was observed (data not shown). Resistance to antibiotics

is a fundamental pharmacokinetic characteristic of probiotic bacteria and implies that the probiotic can be co-administered with an antibiotic drug. As a result, probiotic microorganisms remain viable and help restore the intestinal microflora more quickly **[21]**. According to Bettache *et al*. **[23]**, *Leuconostoc mesenteroides subsp. dextranicum*, *Leuconostoc citreum* and *Leuconostoc lactis* were sensitive to ampicillin, tetracycline, erythromycin and streptomycin. On the other hand, Benmechernene *et al*. **[15]** reported that *Leuconostoc mesenteroides* strains were resistant to ampicillin, tetracycline, erythromycin and streptomycin.

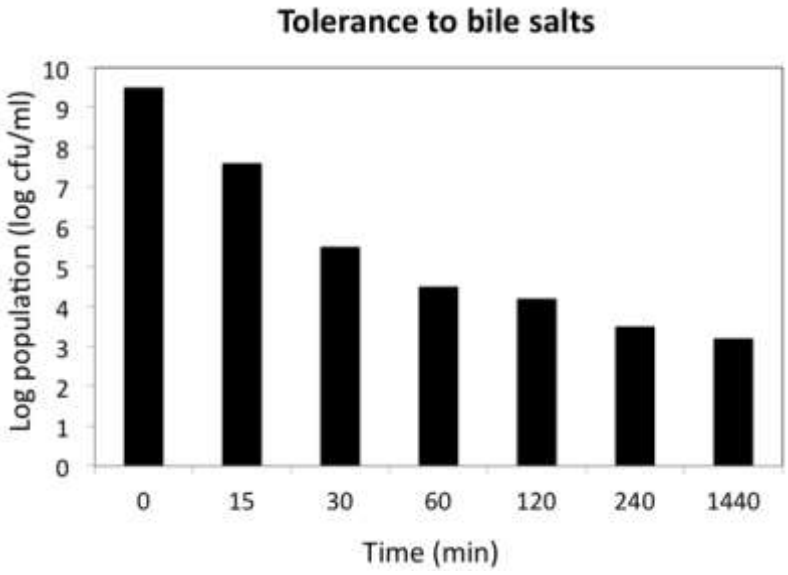

Figure 1: Survivor bar chart of the isolate

Table 1: Microscopic, physiological and biochemical characterization of isolate

Tests	Results
Microscopic	**Coccobacilli**
Catalase	**-**
Gas from glucose	**+**
4% NaCl	**+**
6.5% NaCl	**-**
Growth at 4 C	**-**
Growth at 45 C	**-**
Arabinose	**-**
Maltose	**+**
Rhamnose	**-**
Manitol	**-**
Sorbitol	**-**
Lactose	**+**
Sucrose	**+**
Ribose	**-**

CONCLUSION

A lactic acid bacterium was isolated from

dairy effluents of Mauritius. To ascertain its probiotic potential, the isolate was subjected to a panel of microscopic, physiological, biochemical and viability tests. The isolate was found to be a catalase negative, heterofermentative, gram positive coccobacillus that was able to ferment glucose as well as disaccharides such as lactose, sucrose and maltose. The isolate was tolerant to low pH (pH = 3), presence of bile salts (0.3%) as well as moderate concentrations of NaCl (4%). In addition, its resistance to commonly used broad-spectrum antibiotics as well as its ability to elaborate antibacterials further corroborated its probiotic value. Sequencing and PCR confirmation revealed this isolate to be *Leuconostoc mesenteroides.* Further investigation including *in vivo* studies to evaluate its potential health benefits and fermentation studies to assess its technological characteristics for applications as novel probiotic starters are needed. This ground-breaking study carried out in Mauritius has shown that dairy wastes can be exploited for isolation of commercially valuable probiotic microorganisms.

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