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Isolation and antimicrobial activity of lactic acid bacteria isolated from pineapple and watermelon

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Abstract

Probiotics are living microorganism which when consumed in adequate amount provide health benefit to the host, examples are lactic acid bacteria which are gram positive organisms that can withstand different environmental stress during production and storage of products containing them. This study aims to evaluate probiotic potential of lactic acid bacteria (LAB) strains isolated from pineapple and water melon. The materials used in the evaluation were properly sterilized including the different media like De-man Rogosa & Sharpe agar (MRS), MRS broth for the isolation of LAB by streaking method, nutrient agar, and Mueller Hinton agar for the antibiotic susceptibility test using Disc diffusion method. Out of 168 bacteria counted on plate count agar, a total of six strains were screened based on their cultural and biochemical characteristics. The fruit isolates were screened for antibacterial activity and for their antibiotics susceptibility; the six isolates showed antibacterial behaviour towards both gram-positive and gram-negative human pathogens (*Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Corynebacteria* spp.), while some showed no zone of inhibition. The antibiotic susceptibility test was also promising with P1 showing 31 mm against Amoxiclav. Molecular identification analysis (16sRNA sequencing) revealed that the isolates were different species belonging to the same group, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Weissella cibaria*. Conclusively, these results demonstrate that bacteria isolated from fruit samples have interesting properties and could potentially be used as probiotics and in the preparation of functional fruit products.

Keyword: Fruits; Lactic acid bacteria; Probiotics; Pathogens

1. Introduction

The concept of probiotic was first introduced by Elie Metchnikoff, who observed that the consumption of fermented milk could reverse putrefactive effects of the gut microflora (Peera and James 2012). The application of probiotic bacteria in food for promoting health benefits has been carried out for 20 years. The increasing demand in this functional food is a response to the consumer demand for health food options (Menrad, 2002). The term probiotic was defined as “a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance” (Aslam and Qazi, 2010). Probiotic bacteria may produce various compounds, which are inhibitory to the pathogen’s growth, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. The organic acids not only lower the pH, thereby affecting the growth of the pathogen, but they can also be toxic to the microbes (Tambekar and Bhutada, 2010). There is increasing evidence that probiotics are beneficial in gastrointestinal disturbances, such as diarrhoea, dysentery, typhoid etc. (Tambekar and Bhutada, 2010). It is important to underline when considering the effectiveness and biological activity of probiotics, prebiotics or their combination (synbiotics) that they are food products and not drugs.

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Nowadays there are many different probiotic foods and feeds, and in all products probiotic bacteria have to cope with different stress situations during the processing and storage such as temperatures (high and low), water activity (a_w), acidity, oxygen, and presence of other microorganisms or harmful chemicals. Of course, before the bacteria strains can be used, they have to be capable of being stored and remaining viable after storage. Usually either freezing or drying is used for stocking. It is also important to know how the process and storage may affect the strains; not only should they survive each stress situation, but they should still be viable and able to multiply (Stolaki *et al.*, 2012). Furthermore, in many cases, their effects are mainly prophylactic in nature, rather than therapeutic, i.e. preventive rather than curative (Stolaki *et al.*, 2012).

Lactic acid bacteria were referred to as probiotics in scientific literature by (Ram *et al.*, 2013). Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the LAB and bifidobacteria. Within the group, LAB species are the most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics; the antagonistic activity of which is known to inhibit a large number of enteric and urinary pathogenic bacteria (Hutt, 2006).

Lactic acid bacteria (LAB) can be found in fermented food, plants, fruits and berries. Usually LAB live in nutritionally rich niches, and so LAB can be found in many foods, but they are also part of the normal human gut flora. LAB are a group of bacteria which share several common characteristics, e.g. metabolism and physiology. There is no one way to describe lactic acid bacteria. There are some general descriptions for LAB, which are good for many genera. Such descriptions are accurate in normal situation. A “typical” lactic acid bacterium is a Gram-positive, non-respiring, non-spore forming, coccus or rod. They are catalase-negative, acid-tolerant, and fastidious, and their major end product from fermentation of carbohydrates is lactic acid. Typical LAB are aero-tolerant anaerobic.

LAB can be divided into two groups by the difference in the way they metabolize glucose. The first convention is the use of glycolysis (Embed- Meyerhof pathway), which produces 2 moles of lactic acid from 1 mole of glucose. This type of fermentation is called homofermentative. The other way is heterofermentative, where the LAB convert glucose to lactic acid, carbon dioxide and ethanol or acetic acid. These pathways need standard conditions, a non-limited concentration of sugar and other growth factors (e.g. amino acids, vitamins and nucleic acid precursors) and limited oxygen availability (Axelsson, 1998). The fermentation pathway of LAB can be tested with a gas (CO_2) production test, which can be performed e.g. by using the Durham tube (Endo *et al.*, 2009).

Fruit and vegetable juices have been suggested as an ideal medium for cultivating probiotic microorganisms (Bracket and Splittstoesser, 2001). Fruit and vegetable juices have functional health ingredients because they inherently contain beneficial nutrients (e.g., vitamins), also rich in antioxidants, dietary fibers and minerals (Yoon *et al.*, 2006) and may be considered as an alternative vehicle for delivery and incorporation of probiotics into human intestine. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population. They have taste profiles that are pleasing to all age groups and are perceived as healthy and refreshing (Luckow and Delahunty, 2004). This is because they are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics. Fruit and vegetable juices contain high amounts of sugars which could encourage probiotic growth. For these reasons, fruit and vegetable juices should be examined for their ability to support probiotic and prebiotic delivery in humans.

Watermelon juice is a healthy drink which is rich in lycopene, minerals and vitamins such as A, B and C. Regular consumption of water melon juice can increase blood concentration of lycopene and beta-carotene (Edwards *et al.*, 2003). Studies suggest that these antioxidants may have protective effects against heart disease and certain cancers, such as prostate, bladder, and cervical cancer (Edwards *et al.*, 2003). Tomato juice contains water (93.1%), carbohydrate (4.89%), lycopene, vitamins, minerals and is low in protein and fat which made Tomato juice as one of the healthy beverages. Nowadays, food is no longer considered by consumers 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. Currently, the largest segment of the functional food market is dominated by healthy food products targeted towards improving the balance and activity of the intestinal microflora.

Consumption of food containing live bacteria is the oldest and still most widely used way to increase the number of advantageous bacteria called “probiotics” in the intestinal tract. Noteworthy, there are a large number of probiotic foods which date back to ancient times which are mostly originated from fermented foods as well as cultured milk products]. The quest to find food ingredients with valuable bioactive properties has encouraged interest in lactic acid bacteria (LAB) with probiotic attributes such as antimicrobial activity against pathogenic microorganisms, antiviral activity, anti-yeast property, antimutagenic, antiplatelet aggregation, and antioxidant attributes etc. In general, it is believed that

probiotics help keep up the balance between harmful and beneficial bacteria in the gut thus maintaining a healthy digestive system. Thus, this study aimed at evaluating the probiotic potential of lactic acid bacteria isolated from pineapple and watermelon.

2. Material and methods

2.1. Sample collection

Fresh Pineapple and Watermelon were purchased from a market in Ado Ekiti. The fruits were washed with sterile distilled water, chopped with sterile cutter in small pieces and 10 g was weighed and was left to ferment for 24 – 72hrs, 10mls of the juice from the fermented sample was dissolved into 90 ml of MRS broth (Trias *et al.*, 2008).

2.2. Isolation and purification of lactic acid bacteria from the fruit samples

After dissolving the fruit juice sample into MRS broth in separate tubes, the tubes were shaken to mix and were incubated at 37 °C for 24 hrs in an aerobic condition. Serial dilution technique was done to reduce the microbial load from the fermented juice, 10ml from the fermented juice was suspended and agitated in 90 ml of distilled water to form a microbial suspension. Serial dilution of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} were made by pipetting 10ml into 90ml water blanks respectively.

2.3. Inoculation on Media

10^{-4} of the serial dilution was inoculated to De-Man Rogosa & Sharpe agar (MRS) plates and Nutrient agar plates. 0.5ml was transferred to the center of a sterile petri dish. The sample was spread and mixed with nutrient agar using spread plate method and was incubated at 37 °C for 24 hrs for bacterial growth. The plates were observed for appearance of colonies and number of colonies produced on each plate of different dilution was recovered (Hoque *et al.*, 2010). Samples from the dilutions were also streaked on plate count Agar (PCA) to count the total number of colonies that can be found in each fermented sample, Bacteria culture were purified by streak plate method on MRS agar and incubated at 37 °C for 24hrs for isolation and transferred to MRS agar slants and then maintained in refrigerator at 40 °C till further analysis.

2.4. Purification of Bacteria

The bacterial isolated were purified by streaking each type of the colonies onto fresh nutrient agar and MRS agar using inoculating loop with constant flaming, incubation of the plates was done for 24hours at 37 °C. Smears were made on clean glass slides from the growths and gram staining was carried out as explained below. The stained slides were observed under the microscope for purity. The purified isolates were then placed on agar slants incubated at 37 °C for 24 hours and kept in the fridge until required for further analysis.

2.5. Standardization of test microorganisms

The tested microorganisms were standardized by using 0.5 McFarland standards. McFarland Standard was used as reference to adjust the turbidity of microbial suspensions so that their number will be within a given range. 0.5 McFarland gives approximate cell density of 1.5×10^8 CFU/ml, having absorbance of 0.132 at wavelength of 600 nm. The microbial suspensions were prepared in their respective sterile nutrient broth and are compared either visually or by measuring the absorbance with that of the standard (Andrews, 2001).

2.6. Screening of isolated bacterial cultures for antimicrobial activity

For screening of isolated bacterial cultures, these cultures were inoculated to MRS broth incubated at 37 °C for 24-48 hrs on shaken to carry out the fermentation process. After incubation, 2 ml of each fermented culture broth and supernatant was taken to test the antimicrobial activity by agar well diffusion method. The antimicrobial properties was tested against A total of nine human pathogenic strains of both fungi such as *Candida albicans* and bacterial strains (Gram-negative and Gram positive namely *E. coli*, *Salmonella enteric*, *K. pneumonia*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Corynebacterium spp.*). Each isolated culture was screened against every test microorganism. An overnight culture of pathogens grown in their respective medium at 37 °C was diluted to a turbidity equivalent to that of a 0.5 McFarland standard (Khunajakr *et al.*, 2008).

2.7. Antibiotic susceptibility

The antibiotic susceptibility of isolated LAB was assessed using antibiotic discs diffusion method on Mueller Hilton agar plates. Broth cultures of LAB was prepared using MRS and adjusted to 0.5 McFarland standards. A 100 µl suspension of

freshly grown bacterial cultures was spread on Mueller Hilton agar. The antibiotic discs were placed on the surface of agar and the plates were incubated at 37 °C for 48 hrs. Susceptibility pattern was assessed using antibiotics like Penicillin G, Cephalothin, Oxacillin, Clindamycin, Erythromycin, and Amoxyclav.

2.8. PCR amplification using lactic acid bacteria specific primers

Polymerase chain reaction was carried out to identify lactic acid bacteria using the primer pair BSF-8(AGAGTTTGGATCCTGGCTCAG) and BSR- 534(ATTACCGCGGCTGCTGGC). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM MgCl₂, 200 µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture.

Thermal cycling was conducted in a Peltier thermal cycler (PTC100) (MJ Research Series) for an initial denaturation of 95 °C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95 °C; 1 minute at 58 °C and 1 minute 30 Seconds at 72 °C. This was followed by a final extension step of 10 minutes at 72 °C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard.

3. Results

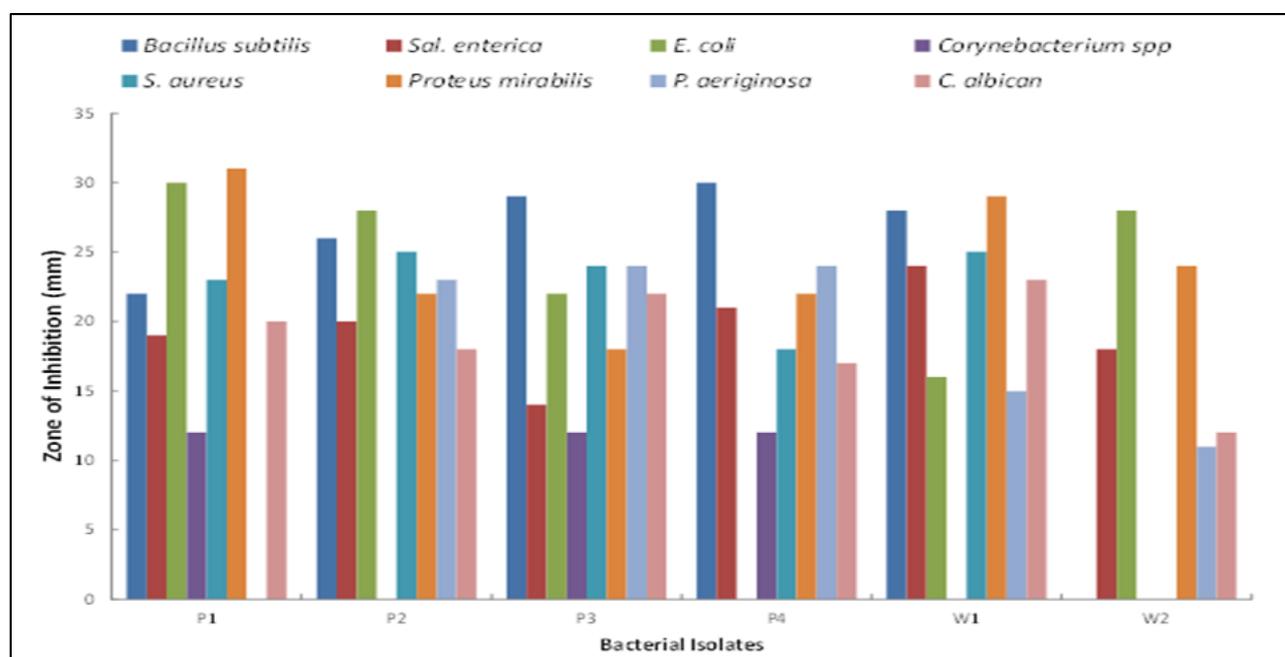


Figure 1 Antimicrobial activity of LAB isolated from fruits

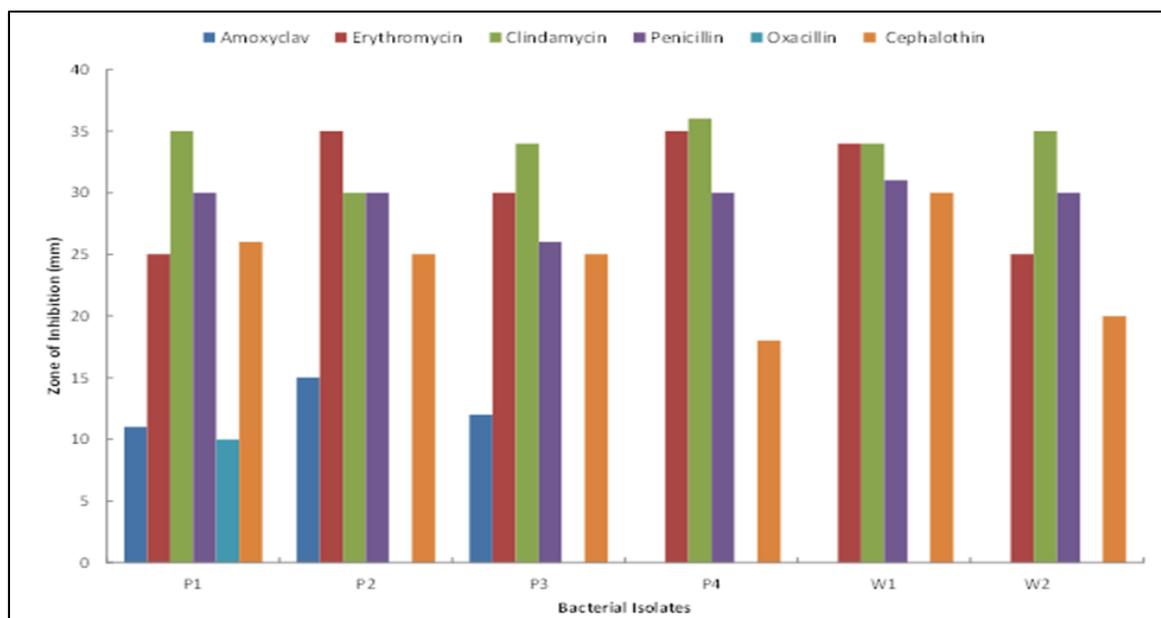
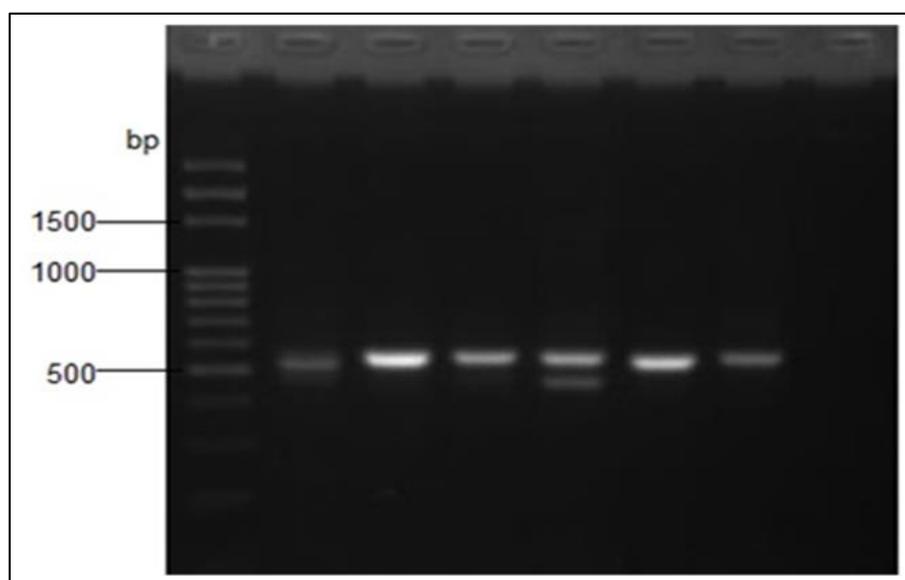


Figure 2 Antibiotic susceptibility of the isolates



The Real-Time was used to distinguish the closely related species of the lactobacillus species. The expected size of the amplicons centered around 500 bp

Figure 3 Real-Time PCR amplification patterns

4. Discussion

A total of 168 bacterial cultures were isolated from fruit samples. Out of which 6 isolates were tested, some isolates were found to exhibit antimicrobial activity against indicator strains as shown in Figure 1. The isolates P1 and P3 showed inhibitory activity against all eight tested pathogenic strains whereas isolates P2, W1 and W2 exhibited activity against 7 tested strains out of 8 and W3 of the isolates showed activity against 5. On the basis of maximum zone of inhibition and inhibition against almost all tested microorganisms occurred with the eight isolates (P1, P2, P3, W1, W2 and W3) and the 6 out of 20 isolates were selected for further evaluation of probiotic properties.

Out of the 6 isolates, P1 isolate showed maximum zone of inhibition against *Proteus mirabilis* of 31mm followed by *Escherichia coli* (30 mm), *P. aeruginosa* with 27 mm, *S. aureus* 23 mm and *Bacillus subtilis* (22 mm) *Candida albicans* (20

mm), *Staphylococcus aureus* (19 mm) and minimum against *Corynebacterium* spp. (12 mm); P2 isolate exhibited maximum zone of inhibition against *E. coli* of 28 mm, followed by *B. subtilis* (26 mm), *S. aureus* (25 mm), *P. aeruginosa* (23 mm), *P. mirabilis* (22 mm), *Sal. enterica* (20 mm), and minimum against *C. albicans* with 18 mm) and no activity against *Corynebacterium* spp.

P3 isolate showed maximum zone of inhibition against *B. subtilis* of 29 mm, followed by *S. aureus* (24 mm), *P. aeruginosa* (24 mm), *E. coli* (22 mm), *C. albicans* (22 mm), *P. aeruginosa* (18 mm) and minimum against *Sal. enterica* (14 mm) and *Corynebacterium* spp. with zone of inhibition 12 mm.

Isolate W1 showed maximum zone of inhibition against *Bacillus subtilis* (30mm), *P. aeruginosa* with zone of inhibition of 24mm, followed by *Proteus mirabilis* (22 mm) and *Sal. enterica* (21 mm), *S. aureus* (18 mm), *C. albicans* (17 mm) and minimum against *Corynebacterium* spp. with zone of inhibition of 12 mm in diameter but did not show inhibition against *E. coli*. W2 showed maximum inhibition against *Proteus mirabilis* with zone of inhibition of 29mm, followed by *B. subtilis* (28 mm), *S. aureus* (25 mm), *Sal. enterica* (24 mm), *C. albicans* (23 mm), *E. coli* (16mm) and minimum against *P. aeruginosa* (15 mm) but no inhibition against *Corynebacterium* spp. was observed; W3 showed maximum inhibition against *E. coli* with zone of inhibition of 28 mm, followed by *P. mirabilis* (24 mm), *Sal. enterica* (18 mm), *C. albicans* (12 mm), and minimum against *P. aeruginosa* (11 mm) but failed to show inhibition against *B. subtilis*, *Corynebacterium* spp. and *S. aureus*

In this study the isolated lactic acid bacterial cultures inhibited both the tested fungal strains and the bacterial strains. Out of tested bacterial strains gram positive bacteria were more sensitive than gram negative bacteria.

The antibacterial activity may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compounds (Bezkorvainy, 2001; Tambekar *et al*, 2009).

The antibiotic susceptibility pattern of the Lab isolates as shown on Figure 2. It shows that P1, P2 and P3 are susceptible to Amoxyclav while P4, W1 and W2 were resistance. All the isolates were susceptible to Erythromycine, Clindamycin, Penicillin, and Cephalothin with the zone range measuring from 18 as minimum and 35 as maximum. Oxacillin is an exceptional case as P1 is only susceptible with 10mm zone of inhibition while others were resistance to it.

The isolates were taken for molecular analysis to authenticate the identity of the isolates after biochemical test and the result of the 16sRNA sequence is as shown above confirming the names to be P1 as *Lactobacillus fermentum*, P2 is *Lactobacillus platarum*, P4 is *Weissella cibaria* and W2 is *Lactobacillus fermentum*. The PCR result shows all the isolates belong to the same family and are all lying around 500bp (Figure 3).

5. Conclusion

It can be concluded that lactic acid bacteria has probiotic potential, and can show antimicrobial activity against some pathogenic organisms. The fruits can serve as good prebiotics due to their rich fibre content and therefore frequent consumption of fruits should be encouraged as sources of vitamins. The lactic acid bacteria are also an added advantage. The isolates showed resistance in acidic and bile salt media; moreover, they also demonstrated antibacterial activity towards both gram-positive and gram-negative pathogenic microorganisms. These features make the isolates suitable candidates for probiotic use. For their potential to be fully exploited, their safety should be tested; this will be indeed done in the future.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest exists.

References

- [1] Edwards AJ, Vinyard BT, Wiley ER *et al.* (2003): Consumption of watermelon juice increases plasma concentrations of lycopene and beta-carotene in humans. *J Nutr*; 133(4): 1043-50. 2003.

- [2] Peera H. and James V. (2012). Effect of probiotics on gut microbiota: mechanism of intestinal immunomodulation and neuromodulation. *Therap Adv Gastroenterol* 2013 Jan 6(1) : 39-51
- [3] Ram Kumar Pundir, Satish Rana Neha kashyap and Amandeep Kaur. Probiotic Potential of Lactic acid Bacteria Isolated from food Samples – an in Vitro study. *J App Pharm Sci.* 2013; 3 (03): 085- 093.
- [4] Axelsson, L., (1998). Lactic Acid Bacteria: Classification and Physiology. In: *Lactic Acid Bacteria, Microbiology and functional Aspects.* Ed. S Salminen and A von Wright, Marcel Decker Inc, New York, USA.
- [5] Brackett, R. E. and Splittstoesser, D. F. (2001): Fruits and vegetables. In F. P. Downes, K. Ito (eds.), *Compendium of Methods for the Microbiological Examination of Foods.* Washington, DC: American Public Health Association, 2001, pp. 515–520.
- [6] Yoon, K. Y., Woodams, E. E., Hang, Y. D. Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology* 97(12) (2006): 1427–1430.
- [7] Luckow, T., Delahunty, C. Which juice is healthier? A consumer study of probiotic non-dairy juice drinks. *Food Quality and Preference* 15(7–8) (2004): 751–759.
- [8] Tambekar, D. H. and Bhutada, S. A. (2010): Studies on antimicrobial activity and characteristics of bacteriocin produced by *Lactobacillus* strains isolated from milk of domestic animals. *The Internet J Microbiol.* 8: 1-6
- [9] Trias, R., Baneras, L., Badosa, E. and Montesinos, E. Bioprotection of Golden delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *Int J Food Microbiol.* 2008; 123:50-60.
- [10] Hoque, M. Z., Akter, F., Hossain, K. M., Rahman, M. S. M., Billah, M. M. and Islam, K. M. D. Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus* Spp. From Selective Regional Yoghurts. *World Journal of Dairy & Food Sciences.* 2010; 5 (1): 39-46.
- [11] Khunajakr, N., Wongwicharn, A., Moonmangmee, D. and Tantipaiboonvut, S. Screening and identification of lactic acid bacteria producing antimicrobial compounds from pig gastrointestinal tracts. *KMITL Sci. Tech. J.* 2008; 8:8-17.