IN VITRO STUDY ANALYSIS OF ANTIMICROBIAL PROPERTIES OF LACTIC ACID BACTERIA AGAINST PATHOGENS

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(Received on Date: 15th December 2015             Date of Acceptance: 10th March 2016)

ABSTRACT

In recent years, interest in Lactic Acid Bacteria (LAB) has grown considerably due to their potential fermentation activity in the production of foods and for the vast array of health benefits they confer. The viability of probiotic bacteria is an essential factor for human immunity. This depends on the ability of the bacteria to survive adverse conditions in the gastrointestinal tract. A well-known attribute of LAB is its ability to produce certain antimicrobial metabolites such as organic acids (lactic acid and acetic acid), hydrogen peroxide and bacteriocins that are known to inhibit pathogenic bacteria and prevent their colonizing in the gut. Therefore an attempt was made to study and elucidate the antimicrobial activity of Lactobacillus bulgaricus, Lactobacillus casei and Lactobacillus bulgaricus with Streptococcus thermophilus. Results indicate mix culture of L.bulgaricus and S. thermophilus had the highest antimicrobial activity against S. aureus. L. bulgaricus had high antimicrobial activity against S. aureus and lowest against E.coli. Similar antimicrobial efficiency was seen in L.casei. These results suggest that LAB could be useful as a probiotic to increase immunity of a host through dietary supplementation.

Key words: L. bulgaricus, S. thermophilus, L. casei, S. aureus, E. coli, Probiotics, Pathogens

Lactic acid bacteria (LAB), antimicrobial property

Number of Tables : 2 Number of Figures : 4 Number of References : 32
INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria that produce lactic acid as their main fermentation product (Mathur, 2005). Typical LAB members are Gram-positive, facultative anaerobic, catalase negative organisms with low G+C content. Most LAB have a long history of being consumed as part of traditional fermented foods and have been awarded the status of “Generally Regarded As Safe” (GRAS) by the Food and Drug Administration (FDA) (Ammor et al., 2007). The LAB group comprises the genera Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Pedicoccus, Aerococcus, Alloicoccus, Dolosigranulum, Enterococcus, Globicatella, Lactospaera, Oenococcus, Carnobacterium, Tetragenococcus, Vagoccus and Weissella (Ko and Ahn, 2000). Lactobacilli are naturally present or deliberately added as starter cultures in unpasteurized milk and dairy products such as cheeses, yogurts and fermented milks (Coeuret et al., 2004). Yoghurt is a common product in which probiotic bacteria can be delivered to the human lower gut is made by fermentation of milk with the starter cultures Lactobacillus delbrueckii subspecies bulgaricus and Streptococcus thermophilus (Hamilton-Miller, 2004). LAB offers the host protection against disease, and promotes normal intestinal function (Fooks and Gibson, 2002). These microorganisms survive the passage through the gastrointestinal tract and eventually establish in the colon. However, they must be taken regularly and at sufficiently high levels to avoid washout and to ensure sustained benefits (Peres et al., 2012). Their benefits are related to the prevention of growth of harmful bacteria by competitive exclusion and by the production of organic compounds. Associated effects of probiotics include prevention and treatment of diarrhoea, alleviation of lactose intolerance, immune modulation and prevention or alleviation of allergies in children (Erkkilä and Petäjä, 2000). Lactobacilli have been demonstrated to have numerous potentially important benefits in terms of gut health and immunity. They can stimulate immune mechanisms at the intestinal level, increase immunoglobulin secretion, enhance antigen presentation and macrophage activation, and inhibit mucosal attachment of pathogens. (Tanriover et al., 2012). Their benefits are related to the prevention of growth of harmful bacteria by competitive exclusion and by the production of organic compounds. Associated effects
of probiotics include prevention and treatment of diarrhoea, alleviation of lactose intolerance, immune modulation and prevention or alleviation of allergies in children (Erkkilä, and Petäjä, 2000). Translocation of viable or probiotic bacteria in minute amounts constitutes a physiologically important boost to the immune system (Lichtman, 2001). Immunomodulatory and immunostimulatory functions (Fooks and Gibson, 2002). Management of inflammatory bowel diseases (Gill and Guarner, 2004), treatment of infections during pregnancy, management of allergic diseases, control of antibiotic-related diarrhoea and prevention of urinary tract infections, amongst others (Jayne et al., 2014), alleviation of lactose intolerance (Marteau et al., 1990). It is speculated that inflammation associated with rheumatoid arthritis may be modulated by the use of probiotics (Marteau et al., 2001). The beneficial effects of probiotics depend on their colonization of the gut and their effect on harmful bacteria, for which certain functional properties are necessary (Hyronimus et al., 2000). They are capable of adhering to human epithelial cells; they prevent colonization by pathogenic bacteria, either by immune exclusion, competitive adhesion or synthesis of antimicrobial substances (Casula and Cutting, 2002; Hyronimus et al., 1998).

As stated the pros of probiotics depends on the efficiency to tolerate and colonize within the gastrointestinal tract. Therefore the main aim of the current study was focused on the antimicrobial activity of Lactobacilli.

**MATERIALS AND METHODS**

**CULTURE MAINTENANCE**

L. bulgaricus and S. thermophilus were procured from National Dairy Research Institute, Bengaluru. It was maintained in skim milk, incubated at 42˚C for 4 hours for S. thermophilus and 6 hours for L. bulgaricus and then refrigerated at 4˚C. Subculturing was done every 7 days. L. casei, isolated from Yakult was maintained in skim milk, incubated at 37˚C for 5 hours and then refrigerated at 4˚C. Subculturing was done every 7 days.

Escherichia coli was isolated from sewage using Eosin Methylene Blue agar (EMB agar) by spread plate method. Colonies with green metallic sheen were selected and sub cultured in Nutrient agar at 37˚C for 24 hours and then refrigerated at 4˚C. Subculturing was done every 7 days. Staphylococcus aureus was procured from Microbial Type Culture Collection, Chandigarh (MTCC number 3160) and was sub cultured in Nutrient agar at 37˚C for 24 hours and then refrigerated at 4˚C. Subculturing was done every 7 days.

**ANALYSIS OF ANTIMICROBIAL ACTIVITY OF LAB**

**Preparation of cell free extract**

The selected LAB species were inoculated to 50ml MRS broth and incubated at 37˚C for 4 and 18hrs. It was centrifuged separately at 10,000 x g for 15 minutes. The supernatant was collected and passed through 0.20 μm sterile syringe filter. The cell free supernatant broth was collected for the
Manuel et al., antibacterial study against selected pathogens (Asth a et al., 2012).

**Antibacterial activity test by agar well diffusion method**

The agar well diffusion method was used to determine the antibacterial property of the LAB. A 24 hr culture of the pathogens (E. coli and S. aureus), grown in nutrient broth at 37°C was used. A lawn of the indicator strain was swabbed on nutrient agar plates. The plates were allowed to dry and a sterile cork borer of diameter (5mm) was used to cut uniform wells in the agar. Each well was filled with 60 µL culture free filtrate and cell suspended in MRS broth obtained from the LAB isolates after 4 hrs and 18 hrs incubation. After incubation at 37°C for 24 hrs, the plates were observed for a zone of inhibition (ZOI) around the well (Asth a et al., 2012). T– test was carried out to determine the significance of variation in anti-microbial activity between cell suspension and cell free extract.

**RESULTS AND DISCUSSION**

An essential condition for LAB with probiotic activity is the productive capacity of inhibitory substances that antagonize pathogenic strains (Nemcova, 1997). The antimicrobial effect exerted by LAB is due to the production of lactic acid and reduction of pH, and acetic acid, diacetyl, hydrogen peroxide, fatty acids, aldehydes, bacteriocin and other compounds (Daeschel, 1989; Jay, 1982).

The agar well diffusion method was used to assess the antibacterial activity of the selected LAB. Their antibacterial properties were tested against pathogenic bacteria namely E. coli and S. aureus (Tables 1 & 2, Figures 1, 2, 3, & 4). The antimicrobial activity was highest in the cell suspension in MRS after 18 hrs incubation. Results indicate that S. thermophilus with L. bulgaricus had the highest antibacterial property, was highest against S. aureus with a ZOI of 10.5±0.35 mm and for E.coli. 4±0mm. L. bulgaricus also showed antibacterial property against all tested pathogens with its activity being highest against S. aureus (10 ± 0 mm) and least against E. coli (3.5 ± 0.35 mm). L. casei also showed antibacterial property against all tested pathogens with its activity being highest against S. aureus (10 ± 0 mm) and least against E.coli (4.0 ± 0 mm). As a part of statistical analysis, the Student T-test showed there is a significant variation in anti-microbial activity between cell suspension and cell free extract except for S. aureus 4 hours incubation ( *p≤ 0.05). Current finding is similar to the results reported by Gilliland and Speck (1977), that lactobacilli showed stronger antibacterial properties against Gram positive bacteria (S. aureus and Clostridium perfringens) than Gram negative bacteria (E. coli and S. typhimurium). Earlier reports (Tagg et al., 1976, Daeschel and Klaenhammer et al., 1985, Sanni et al., 1999) have shown that some bacteriocins produced by Gram-positive bacteria have a broad spectrum of activity.
Manuel et al.,

Fig 1: Antimicrobial activity of a cell suspension against *S. aureus* after an 18hr incubation period.

Fig 2: Antimicrobial activity of a cell free extract against *S. aureus* after an 18hr incubation period.

Fig 3: Antimicrobial activity of a cell suspension against *E. coli* after an 18hr incubation period.
Fig 4: Antimicrobial activity of a cell free extract against E.coli after an 18hr incubation period.

Table 1: Antimicrobial activity of LAB after 4 hours incubation

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<tr>
<th>Sample</th>
<th>Zone of Inhibition (in mm)</th>
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<tr>
<td></td>
<td>Cell suspended in MRS: S. aureus</td>
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<tr>
<td>L. bulgaricus</td>
<td>8.5±0.35</td>
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<tr>
<td>S. thermophilus + L. bulgaricus</td>
<td>8.5±0.35</td>
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<tr>
<td>L. casei</td>
<td>8.5±0.35</td>
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Table 2: Antimicrobial activity of LAB after 18 hours incubation

<table>
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<tr>
<th>SAMPLE</th>
<th>Zone of Inhibition (in mm)</th>
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<tr>
<td></td>
<td>Cell suspended in MRS: S. aureus</td>
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<tr>
<td>L. bulgaricus</td>
<td>10±0</td>
</tr>
<tr>
<td>S. thermophilus + L. bulgaricus</td>
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<td>L. casei</td>
<td>10±0</td>
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ACKNOWLEDGEMENT

We would like to extend our gratitude to Dr. Sr. Arpana, Principal, Mount Carmel College (Autonomous) for all her encouragement and support. We would also like to thank Ms. Usha, Research Assistant, Centre for Scientific Research and Advanced Learning for her support during the work.

REFERENCES


KCA2386 isolated from white kimchi. Food Science and Biotechnology, 9(4), 263-269.


