COMPARATIVE STUDY ON GROWTH PERFORMANCE OF CERTAIN PROBIOTIC STRAINS CULTIVATED IN NATURAL JUICES AND ANALOGOUS SELECTIVE MEDIA

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ABSTRACT

Different Fruits and vegetables juice were evaluated as a conceivable substrate for the cultivation of certain probiotic strains viz; Lactobacillus Acidophilus, Streptococcus Faecalis, Bacillus Mesentricus. All the cultured test probiotic strain under present study showed the presence of more or less comparable growth in all the natural juices. This indicates the possibility that the natural juices may be used as the substrate for probiotic strain. However, the growth of probiotic strain, recorded in its analogous Selective Media exhibited that the GT of all three probiotic strains were shortererits respective selective media and the TVC of probiotic strains were found to be more than TVC recorded in natural juices. This indicates that the selective media are more compatible for the growth and hence Natural Juices shall be modified at nutrition level to achieve the comparable compatibility. However, present investigation shed light on the high possibility of probiotic organism cultivation using natural juices. It was also observed that among all the probiotic cultures tested, the growth of only L.acidophillus was found to be more in Pineapple, Orange, Beetroot, amla and aloe vera juice over other juices and probiotic cultures. Among which the beetroot, amla and aloe vera juice may be the more conceivable substrate for the cultivation of L.acidophilus.

INTRODUCTION

Rendering to Traditional as well as Modern medicinal concept, use of fermented food is the best alternative for the treatment of Malnutrition, Diarrhea, Gastrointestinal diseases. In view of the fact that consumption of fermented food boosts the digestive system by providing an entry route for the common biological agents that contain the beneficial intestinal flora i.e. Probiotic Holzapfel W (2002), Fitzgerald RJ and Nagpal R.et.al (2012). As defined by FAO/WHO (2001), probiotics are live microorganisms as the bacteria and yeast that confer a beneficial health effect on the host if administered in appropriate amounts. Foods which contain probiotics when ingested in sufficient quantities can have specific physiological health benefits such as anti-pathogenic, anti-diarrheal, anti-carcinogenic, anti-diabetic, anti-cholesterol properties etc. to individual’s health.

Lactobacillus & Bifidobacterium are most commonly used Probiotics. Other microorganisms such as yeast; Saccharomyces Cerevisiae & some E. coli & Bacillus species are also used as probiotics. Traditionally, yoghurt and other fermented dairy products are considered as best probiotic carriers, since milk is the ideal source for probiotics growth. However, there is a large variety of fruits and vegetables available locally that could be possible to be exploited as a substrate for probiotics.

Fruit and vegetables provide a range of nutrients and different bioactive compounds including phytochemicals, vitamins, minerals & fibers. Fruit and vegetables juices are the ideal Natural juice as they have high nutritional content, good results in prophylaxis & in treating cardiovascular disease. As the natural juices have a purifying effect, its consumption is good for liver and gall bladder (Segel, 1999). According to the increasing evidences, it is suggested that, a healthy eating practice with increased consumption of plant based foods plays important roles in prevention of chronic diseases, such as heart disease, cancer, stroke, diabetes & aging. (Liu,2004)

Different studies are approved to explore the quality of natural juices as raw materials for the invention of probiotic drinks. Fruits and vegetables juices are rich source of saccharides; they will be served as proper medium to cultivate probiotic lactic acid bacteria to boost the health benefits of food products.

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Additionally, these juices contain vitamins A, C and E, which have a high antioxidant capacity and phenolic compounds. Furthermore, absence of dairy allergens makes fruits and vegetable juices use by certain segments of the population (Luckow and Delahunty, 2004). Taking into consideration that, a novel effort was made through present investigation in order to observe the growth performance of certain probiotic bacteria in different natural juices in comparison with respective selective medium, so that the natural juices may proven a better substrate for lactic acid fermentation.

MATERIALS AND METHODS

Isolation, Identification & Enrichment of probiotic culture

Commercially available probiotic supplement Viabact sachet (USV PVT LTD) was purchased from local retailers. The probiotic (1 g) was added to 9 mL phosphate-buffered saline (PBS) and vortexed thoroughly. Serial 10-fold dilutions were then made in PBS. A 0.5-mL volume of each dilution was inoculated onto deMan, Rogosa, Sharpe (MRS) agar and 2 blood agar plates. The MRS plates were incubated anaerobically at 37°C for 48 hr. using Gas pack (Himedia) for isolation of lactic acid bacteria. Lactic acid bacteria were identified by Gram stain, colony morphology, biochemical characterization and catalase reaction. For the isolation of streptococci, Blood agar plates were incubated aerobically at 35°C and identified by colony morphology on blood agar, Gram stain appearance, biochemical chacterization and catalase reaction. For the isolation of bacilli, blood agar plates were incubated anaerobically at 37°C and were identified by Gram stain, colony morphology, biochemical characteristics and catalase reaction. The identified bacteria viz; Lactobacillus acidophilus, Bacillus Mesentricus & Streptococcus Faecalis were subjected for enrichment in MRS broth & used for further Experimentation. The enrichment broth was subjected for enumeration adopting Standard Plate Count. The incubation was continued till the count reaches Upto 10^7/ml

Sample Collection and Preparation of Substrate

The fruit, fruit vegetable and herbal plant juices were used as natural juices. Fruits and vegetables viz. Orange, Sweet lime, Pineapple, Guava, Papaya, Apple, Sugarcane, Beetroot, Carrot & Tomato as well as Amla and Aloe vera were purchased from local market. The selected fruit, fruit vegetables and herbal plant were washed thoroughly with running tap water, rinsed with distilled water and blotted dry. Then cuts into small pieces, peeled off and seeds are separated manually from the pulp. The juice was then extracted with the help of juicer and then filtered. The filtered juice then sterilized under UV radiation up to 10 min and the sterilized juice was used as the substrate for the fermentation. The above process was followed for each fruit, fruit vegetable & herbal plant under study.

Fermentation

Experiment was carried out in triplicate as batch fermentation (in flasks). Pasteurized juice was inoculated with a 10% (v/v) of Lactobacillus acidophilus, Bacillus mesentricus & Streptococcus Faecalis inoculums separately and incubated at 37°C for 48 hr for fermentation. The above process was followed for each natural juice under study. The cultures of probiotic test organisms were also inoculated into their selective media for the comparative studies.

Effect of fermentation on Growth rate of probiotic bacteria

An aliquot from each juice & selective media of respective probiotic bacteria was taken after fermentation and the turbidity measure at 640 nm for comparative study. Specific growth rate (µ) for each culture was calculated using the equation: µ = (lnD2– lnD1)/ (t2 – t1), where D2 and D1 are the cell densities at times t2 and t1, respectively. Percent increase in generation time was calculated.

Effect of fermentation on the growth of probiotic bacteria

During fermentation growth of organisms in natural juice was measured through plate count technique (Dimitroski et al., 2015) and microbial population was recorded in CFU/ml. Fermentation time which showed maximum population was considered as the optimum fermentation time in further processes

Statistical analysis

The experimental design and statistical analysis were performed using Design Expert Software Version 9.0.4. Analysis of variance (ANOVA) was conducted to determine whether significant effect existed for the factors. Mean Standard deviation and analysis of Microsoft Excel software and variance was used to describe the result. Differences at P < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Wide variety of fruits and vegetables and many strains of probiotic organisms have provided a great opportunity for the development and industrialization of nondairy fermented beverages. Natural juices are suitable for probiotics transfer due to having minerals, vitamins, dietary fiber and antioxidants (Moraru.et.al.2007; Yoon et.al.,2004). Fruit juice could serve as a good medium for cultivating probiotics (Mattila-Sandholmet.al., 2002).Taking this into consideration, in present investigation 12 different natural juices was used as substrate to study the growth performance of three probiotic bacteria namely Lactobacillus acidophilus, streptococcus faecalis & Bacillus mesentricus respectively. The three probiotic bacteria were isolated and identified on the basis of gram staining, biochemical characterization and catalase reaction presents in table no. 1.Three probiotic bacteria were incorporated in each natural juice taken under study & incubated for up to 48 hrs at 37 °C. Before fermentation the pH of all the juices was neutralized (pH 6-7) by NaOH solution. The three probiotic bacteria used in this study showed significant growth in all juices & found capable of utilizing natural juice for lactic acid fermentation without supplementation. Generation time & Total viable count for each probiotic bacteria were presented in Fig.1 & Fig.2, respectively. Among the natural juices used in study Orange, Pineapple, Beetroot, Tomato, Amla and Aloe vera juice showed effective growth of probiotic bacteria. L. acidophilus, streptococcus faecalis, & Bacillus mesentricus, showed maximum growth in selective media as compare with natural juices. Lactobacillus acidophilus required 53 min. to grow in selective media. In case of Aloe vera juice the GT was increases by 7% for L.acidophilus, 12.5% for S.faecalis & 36% for B.mesentricus. In case of Amla and Beetroot juice the generation time was increases by 10% & 13 % for L. acidophilus, 16% & 27% for S.faecalis,42% &48% for B. mesentricus. The generation time was increases by 17% &11
% for *L. acidophilus* 37% & 36% for *S. faecalis*, 49% & 50% for *B. mesentricus* in orange & pineapple juice. Sweettime, guava, papaya, apple, sugarcane, carrot & tomato juice shows the significant percent increase in Generation time for all the three probiotic bacteria represented in fig.2. As expected from the result of total viable counts, Fig.1 shows that the highest total viable count was observed in Aloe vera juice from the cultures of *L. acidophilus* (42.4 × 10⁹ CFU/ml), *S. faecalis* (37.3 × 10⁹ CFU/ml) & *B. mesentricus* (33.2 × 10⁹ CFU/ml). The second highest juice was Amla and Beetroot juice in which the total viable count for *Lactobacillus acidophilus* wasas (6.9 × 10⁹ CFU/ml) & (17.5 × 10⁹ CFU/ml), for *S. faecalis* (3.7 × 10⁹ CFU/ml) & (15.1 × 10⁹ CFU/ml) and for *Bacillus mesentricus* (3.9 × 10⁹ CFU/ml) & (15.3 × 10⁹ CFU/ml) in Amla & Beetroot juice respectively. Orange juice, pineapple juice & tomato juice also showed a good viable cell count. In orange juice the total viable count for *L. acidophilus* (3.5 × 10⁹ CFU/ml), *S. faecalis* (4.0 × 10⁹ CFU/ml) & *B. mesentricus* (3.4 × 10⁹ CFU/ml). In case of pineapple juice the viable count for *L. acidophilus* (5.99 × 10⁹ CFU/ml), *S. faecalis* (3.9 × 10⁹ CFU/ml) & *B. mesentricus* (4.5 × 10⁹ CFU/ml). The Total viable count in tomato juice for *Lactobacillus acidophilus* (3.5 × 10⁹ CFU/ml), *B. clausii* (2.9 × 10⁹ CFU/ml) & *B. mesentricus* (3.1 × 10⁹ CFU/ml) respectively. It is important to have a significant number of viable lactic acid bacteria present in finished end product for maximum health benefits (Shah 2001) Probioticiation of fruit juices is also good for developing health beneficial products particularly for those who are allergic to milk products (Sheehan et al., 2007). *Lactobacillus acidophilus* showed significant viable count in Guava, Papaya, Apple & Carrot juice than other two probiotic bacteria. Natural juices used in study, among which Orange, Pineapple, Beetroot, Tomato, Amla & Aloe vera showed less % increase in Generation time in accordance with selective media & shows significant growth (P < 0.05) of all the three probiotic bacteria. Statistical values were validated by ANOVA analysis and F-test all the values were statistically significant because the calculated F-value were greater than the listed F-value (P < 0.05). Fig. 1 and Fig. 2 shows the graph for % increase in Generation time and Total viable count for all the three probiotic bacteria. The result represented here in agreement with other studies (Yoon et al., 2006 ; Gupta et al., 2010), which suggested that different vegetable matrices could serve as good media for growing probiotics by stimulating their effect resulting in good viable counts. Maximum growth was available at different conditions of viability. Microbial survival in foods is strongly dependant on the food matrix (Shah, 2007).

Table 1 Identification of Probiotic Isolates

<table>
<thead>
<tr>
<th>No.</th>
<th>Probiotic isolates</th>
<th>Growth/C</th>
<th>Shape</th>
<th>Motility</th>
<th>Biocompatibility Test</th>
<th>Catalase test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Lactobacillus acidophilus</em></td>
<td>G+</td>
<td>Rod</td>
<td>Non-motile</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Streptococcus faecalis</em></td>
<td>G+</td>
<td>Rod</td>
<td>Non-motile</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus mesentricus</em></td>
<td>G+</td>
<td>Rod</td>
<td>Motile</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

I: Indole test; MR: Methyl Red; VP: VogesPrausnark; C: Citrate test; +: Positive; -: Negative

**Table 2** Generation Time of *L. acidophilus*, *Streptococcus faecalis*, *B. mesentricus* grown in Selective Media and Natural juices

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Lactobacillus acidophilus</th>
<th>Streptococcus faecalis</th>
<th>Bacillus mesentricus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>Generation time (min.)</td>
<td>% Increase in GT</td>
<td>Generation time (min.)</td>
</tr>
<tr>
<td>Selective media</td>
<td>65</td>
<td>00</td>
<td>35</td>
</tr>
<tr>
<td>Orange juice</td>
<td>64</td>
<td>17</td>
<td>37</td>
</tr>
<tr>
<td>Sweet lime juice</td>
<td>72</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Pine apple juice</td>
<td>60</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>Guava juice</td>
<td>84</td>
<td>36</td>
<td>70</td>
</tr>
<tr>
<td>Papaya juice</td>
<td>89</td>
<td>40</td>
<td>73</td>
</tr>
<tr>
<td>Apple juice</td>
<td>85</td>
<td>37</td>
<td>71</td>
</tr>
<tr>
<td>Sugarcane juice</td>
<td>75</td>
<td>29</td>
<td>65</td>
</tr>
<tr>
<td>Beetroot juice</td>
<td>61</td>
<td>13</td>
<td>48</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>86</td>
<td>38</td>
<td>76</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>63</td>
<td>10.9</td>
<td>49</td>
</tr>
<tr>
<td>Amla juice</td>
<td>59</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>Aloe vera juice</td>
<td>57</td>
<td>7</td>
<td>40</td>
</tr>
</tbody>
</table>

GT: Generation Time

**Table 3** Total Viable Count of *L. acidophilus*, *S. faecalis* and *B. mesentricus* grown in Selective Media and natural juices at stationary phase

**Figure 1** % Increase in Generation time of *L. acidophilus*, *S. faecalis* and *B. mesentricus* grown in natural juice

**Figure 2** Graph % Increase in Generation time of *L. acidophilus*, *S. faecalis*, *B. mesentricus* grown in natural juice
CONCLUSION

From the Results of this study, it was concluded that natural juices could serve as raw material for the growth of probiotic organisms. This study showed that probiotics could grow well in natural juices without any external nutritional supplements. Among the three probiotic bacteria used, lactobacillus acidophilus gives noteworthy result and suitable for used as probiotics cultures for production of healthy beverage. Also result showed that Orange, Beetroot, Amla & Aloe vera juice had the maximum growth during fermentation & maximum total viable count. It could be advocated that natural juice was helpful for developing an appropriate probiotic juice with more health benefits which could be served as a health beverage for vegetarians and consumers who are allergic to dairy products.

Acknowledgement

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Reference


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