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EVALUATION OF PROBIOTIC POTENTIAL OF SELECTED LAB CULTURES

ANITA RAISAGAR[1] AND SANGEETA SHUKLA[2]

Sam Higginbottom University of Agriculture, Technology, and Sciences (SHUATS), Allahabad, U.P., India

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Abstract– To provide a beneficial effect on the host, every potential probiotic culture is expected to tolerate the condition of the GI tract. The ability to tolerate acid, bile salt, NaCl, phenol, and temperature is considered a good indicator for the survival of bacterial culture in the GI tract, and these characteristics are often assessed *in vitro* in the preliminary selection of probiotic culture. In the present study, the probiotic potential of two LAB namely *Lactobacillus casei* and *Bifidobacterium bifidum* was studied. Among the selected LAB one was aerobic (*Lactobacillus casei*) and the other was anaerobic (*Bifidobacterium bifidum*). The studied probiotic potential parameters were bile tolerance, pH tolerance, NaCl tolerance, phenol tolerance, and temperature sensitivity. Both the selected LAB showed survival in all tested concentrations of bile (0.5%, 1.0%, 1.5% and 2%), NaCl (1-10%), and phenol (0.1-0.4%). The growth of selected LAB showed a negative tendency towards increasing concentrations of probiotic potential parameters. In the tested pH ranges *Lactobacillus casei* showed survival in pH ranges from pH 3 to pH 8 while *Bifidobacterium bifidum* showed survival with pH 3 to pH 7. In both cases, the optimum pH for growth was pH 7. Both the LAB was able to grow in all tested temperatures range.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci/rodshaped, catalase-negative fastidious bacteria. These are naturally occurring bacteria and 'Generally Recognized as Safe' (GRAS) because it is non-pathogenic to humans and animals (Patil *et al.*, 2010). There are numerous application areas for use of LAB such as use as a starter culture for the production of functional and fermented food, preserver of foods, and probiotics. According to WHO/FAO/OIE probiotics are defined as live microorganisms that provide health benefits along with nutritional effects to the consumer when ingested in a requisite amount (Joint FAO/WHO/OIE, 2003). LABis divided into five genera, namely, *Streptococcus, Lactobacillus, Leuconostoc, Bifidobacteria,* and *Pediococcus*. Out of which *Lactobacillus* and *Bifidobacteria* are the most common and prominent members of the intestinal microflora categorized under probiotics (Espirito Santo *et al.,* 2003). The beneficial effects of these probiotics have been studied and documented by numerous investigations over many years. During the antibiotic treatment, the balance of intestinal microflora is disturbed which causes other health issues. These probiotic organisms beneficially affect the host by improving the balance of the natural micro flora of the intestine and thus improving the host's immune system. Other health-related benefits of probiotics include management of lactose intolerance, prevention of colon cancer and urogenital symptoms, lowering blood pressure and incidence and duration of diarrhea, and reduction of cholesterol and allergic symptoms(Saarela *et al.*, 2002; Mc Naught and Mac Fie, 2001; Rafter, 2003). Probiotic also inhibits the growth of pathogenic bacteria by producing inhibitory compounds (such as bacteriocins and hydrogen peroxide), alteration of pH values by producing organic acids, and competitive adhesion to the epithelium (Kolida *et al.*, 2006). The important factor for probiotics to exert beneficial effects on consumers or host is their viability and successful transfer through the GI tract. For this purpose, probiotics should have resistance to stressful conditions of the GI tract. The important criteria or characteristics for selection of culture as probiotics are non-virulent, bile and acid-tolerant, multiplication in the GI tract, producing beneficial molecules and nutrients, ability to adhere to the cell surface, susceptibility to antimicrobials, and exhibit antimicrobial activity against human pathogens (Fijan, 2016). In both dairy and non-dairy products probiotics are found. Some examples of dairy products having probiotics include fermented milk, cheese, yoghurt, buttermilk, ice cream, etc., and non-dairy products rich in probiotics are soy-based products, nutrition bars, cereals, kimchi, microencapsulation products, etc. (Kechagia *et al.*, 2013).

Taking into consideration the importance of probiotic properties of LAB, probiotic potentials of two LAB viz., *Lactobacillus casei* and *Bifidobacterium bifidum* were studied in the present work. This study highlights the health-promoting properties and mode of antimicrobial action of selected probiotics. Also, results help in the selection of probiotic cultures as adjunct cultures in the dairy and food industries.

MATERIALS AND METHODS

Procurement and maintenance of selected LAB cultures

Selected LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidum*) were procured from the National Collection of Industrial Microorganisms (NCIM), Pune. *Lactobacillus casei* was revived and maintained in MRS (De Man Rogosa Sharpe) agar slants at 37°C while *Bifidobacterium bifidum* was revived and maintained in MRS agar slants supplemented with 0.05% L-cystein hydrochloride monohydrate in anaerobic condition at 37°C.

Evaluation of probiotic potentials of selected LAB cultures

Selected LAB cultures were examined for their probiotic potential by using bile tolerance, pH tolerance, NaCl tolerance, Phenol tolerance, and Temperature sensitivity. The evaluation procedures of selected parameters are discussed in the following subsections.

Bile Tolerance

To check the bile tolerance, different concentration of bile oxgall *i.e.*, 0.5%, 1.0%, 1.5% and 2% were used. MRS broth prepared with varying concentrations of bile oxgall was inoculated with 24hrs old LAB cultures. MRS broth without bile oxgall was used as control. After incubation, 0.1ml inoculums of each tubewere spread-plated on MRS agarand incubated at 37°C for another 24 hrs. Bile tolerance was estimated after incubation by comparing viable cell counts (CFU/ml) in the control MRS plate and MRS plates with varying concentrations of bile oxgall (Jacobsen *et al.*, 1999).

pH tolerance

MRS broth tubes prepared with varying pH, *i.e.*, pH1 to pH10 were inoculated with 24 hrs old LAB cultures. After incubation at 37 °C for 24 hrs, 0.1ml inoculums of each tube were pourplated on MRS agar and incubated at 37 °C for another 48 hrs. The pH tolerance activity of LAB culture was estimated by comparing bacterial colony counts (CFU/ml) in MRS agar plate with varying pH and control plate (Tambekar and Bhutada, 2010).

NaCl tolerance

MRS broth prepared with different concentrations of NaCl *i.e.*, 1 to 10% was inoculated with LAB cultures. After incubation, 0.1ml inoculums were spread on MRS agar plate from each inoculated tube and incubated at 37 °C for another 48 hrs. CFU/ml count on different agar plates with varying NaCl concentration and control plate was studied to estimate NaCl tolerance (Adebayo-tayo and Onilude, 2008; Hoque *et al.*, 2010).

Phenol tolerance

MRS broth tubes with different concentrations of phenol *i.e.*, 0.1-0.4% were inoculated with 24 hrs old LAB culture. After incubation, 0. 1ml inoculums from each inoculated tube were poured on MRS agar medium and incubated at 37 °C for another 48hrs. Phenol tolerance activity of LAB culture was determined by comparing bacterial colony counts (CFU/ml) in MRS agar plate with varying phenol concentration and control plate (Hoque *et al.*, 2010).

Temperature sensitivity

24hrs old LAB culture was inoculated in MRS broth and incubated at different temperatures, *i.e.*, 25 °C, 30 °C, 37 °C and 40 °C for 48-72 hrs. After incubation, 0.1 ml inoculum from each tube was pour plated on MRS agar. All plates were incubated at 37 °C for another 48 hrs. Temperature sensitivity of selected LAB culture was determined by comparing bacterial colony counts (CFU/ml) in MRS agar plate with varying incubation temperatures (Tambekar and Bhutada, 2010).

RESULTS AND DISCUSSION

Bile tolerance

According to Begley *et al.,* (2005) resistance to Bile salt is one of the important criteria for selecting a LAB as a probiotic strain because it is necessary to perform effectively in the human GI tract. Papadimitriou *et al.,* (2015) suggested a bile salt concentration range of 0.15-0.5% for probiotics. This is the range of the physiological concentrations that are met in the GI tract. In the present study, both selected LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidum*) showed growth in different concentrations of bile. These findings are comparable to a previous study done by Pundir *et al.,* (2013), in which bile salt tolerance in selected LAB isolates was reported at 0.5% to 2.0% concentrations. In another study done by Forhad *et al.,* (2015), all the isolated LAB including *Lactobacillus casei* and *Bifidobacterium* showed tolerance to bile salt concentrations *i.e.,* 0.05%, 0.15% and 0.50%. The ability to tolerate bile salt at a concentration of 0.3% has a physiological significance because the normal concentration of bile salt in the human small intestine is 0.3% (w/v). However, Knarreborg *et al.,* (2003) suggest bile concentration as variable and unpredictable as it changes according to diet composition and has a close relationship with the pancreatic enzyme's secretion. Nevertheless, for investigation of bile tolerance of potentially probiotic *Lactobacillus* culture, research by Sahadeva *et al.,* (2011), Ruiz-Moyano *et al.,* (2008) and Boonkumklao *et al.,* (2005) considered 0.3% bile concentration as the standard level. In the present study, the highest growth was observed in 0.5% bile salt concentrationbut a reduction in the growth of probiotic culture was observed as bile salt concentration increases. Similar reduction trends in cell counts were also found by Huang *et al.,* (2015) with *Lactobacillus* culture for bile salt concentration of 0.03-0.3%. Resistant to bile salts are not dependent on species. Sometimes different strains of the same species showed different resistant patterns as reported in research by Liong and Shah (2005), Mishra and Prasad (2005) and Pennacchia *et al.,* (2004). Bile salt tolerance is a strain-dependent feature and tolerance of species cannot be universal as suggested in the previous study by Begley *et al.,* (2005) and Koll *et al.,* (2008). The counted CFU/ml with different Bile salt concentrations in the selected LAB culture of the present study is shown in Figure 1.

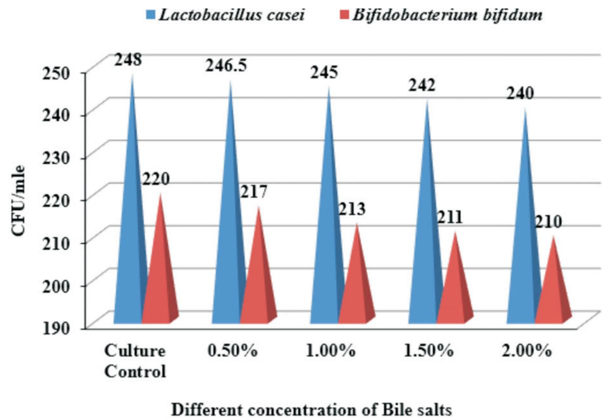

Fig. 1. Bile salt tolerance in selected LAB culture

pH tolerance

According to Musikasang *et al.,* (2009), to classify a LAB as probiotic bacteria, it should resist to low pH environment because it is important for survival and growth in the GI tract to exert probiotic function effectively. In the present study, both LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidus*) were examined for growth in pH values in the range of pH 1 to pH 10. The *Lactobacillus casei* showed growth in the range of pH 3 to pH 8 whereas *Bifidobacterium bifidus* showed growth in the range of pH 3 to pH 7. None of the selected LAB cultures showed growth in pH 1, pH 2, pH 9, and pH 10. The recorded CFU/ml with different pH ranges are shown in Figure 2. Present findings are in accordance with Pundir *et al.,* (2013) results in which they reported that the selected LAB isolates grow in the range of pH 3.5 to pH 7 but failed to grow in the range of pH 1 to pH 3. The pH tolerance in *Bifidobacterium* species is also reported by Awasti *et al.,* (2016), Liu *et al.,* (2013), Hossain *et al.,* (2018), and Afify *et al.,* (2012). The pH tolerance in *Lactobacillus* species at pH range of 3-9 was reported by Hoque *et al.,* (2010); Chakraborty

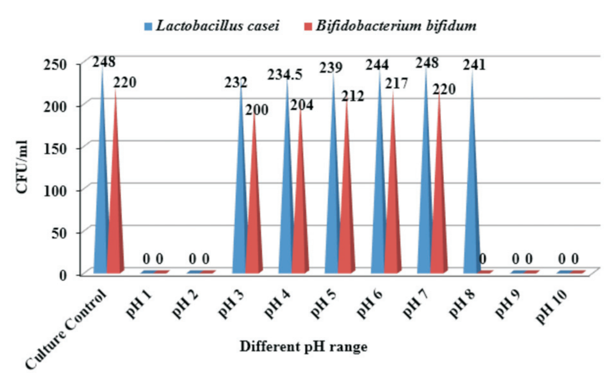

Fig. 2. pH tolerance in selected LAB cultures

and Bhowal (2015), Ilavenil *et al.*, (2015) and Huang *et al.*, (2015). In the present study, in pH 7 the recorded CFU/ml were similar to CFU/ml recorded with culture control. As pH decreases or increases, loss of viability was recorded with both selected LAB cultures. These findings agree with the results found by Dunne and Mahony (2001), where *Lactobacillus* strains were able to retain their viability when exposed to pH values of 2.5–4.0 but displayed loss of viability at lower pH values.

NaCl tolerance

NaCl is an inhibitory substance that may inhibit the growth of certain types of bacteria. Thus, it is an important factor to classify LAB as probiotics because if the LAB is sensitive to NaCl then it will not be able to show its activity or effectiveness in presence of NaCl. In the present study, both selected LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidum*) were examined for NaCl tolerance with 1 to 10% NaCl concentrations. Both cultures showed growth in 1 to 7% concentration while none of the cultures showed growth in 8 to 10% NaCl concentrations. The recorded count (CFU/ml) was found to decrease with increasing NaCl concentrations. The recorded CFU/ml are shown in Figure 3. The current findings agree with the research by Forhad *et al.*, (2015) who reported NaCl tolerance in *Lactobacillus* and *Bifidobacterium* isolates at the concentration range of 1-6%. Their study also concluded that the isolates grow fairly at 7% concentration and failed to grow as concentration further increased. Hossain *et al.*, (2018) also reported the growth of *Bifidobacterium* isolates at 1-5% NaCl concentration while the growth decreases when concentration was increased up to 9%. NaCl tolerance with 1-7% concentrations were also reported in previous research done by Rahman *et al.*, (2016); Saud *et al.*, (2020); Pundir *et al.* (2013) and Adebayo-tayo and Onilude (2008).

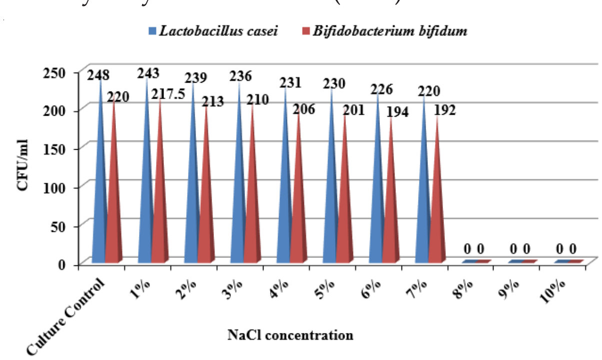

Fig. 3. NaCl tolerance in selected LAB cultures

Phenol tolerance

According to Satyabhama *et al.* (2015) phenol is a toxic metabolite produced by de-amination of some amino acids during disintegration by intestinal bacteria. In the present study, the selected LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidus*) were examined for phenol tolerance with different phenol concentrations viz., 0.1%, 0.2%, 0.3% and 0.4%. Both the cultures showed growth in all tested concentrations. A decreasing trend in CFU/ml count was found with increasing phenol concentrations. The recorded CFU/ml is shown in Figure 4. The findings agree with the research by Forhad *et al.*, (2015) who isolated *Lactobacillus* and *Bifidobacterium* bacteria from buffalo milk samples and reported 0.1% to 0.4% phenol concentration tolerance in all isolates including *Lactobacillus casei, Lactobacillus fermentum, Lactobacillus acidophilus*, and *Bifidobacterium longum*. Research by Rahman *et al.* (2016) found phenol tolerance in LAB with 0.1% to 0.2% concentration but the growth of LAB was reduced as concentration increased from 0.3% to 0.4%. Phenol tolerance at 0.4% in LAB is also reported in the study conducted by Chakraborty and Bhowal (2015).

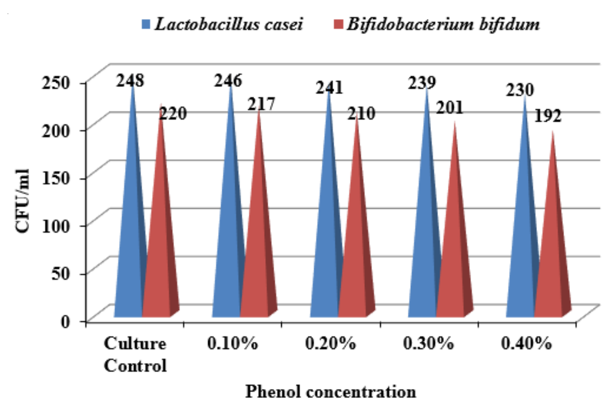

Fig. 4. Phenol tolerance in selected LAB cultures

Temperature Sensitivity

In the present study, both selected LAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidum*) were examined for their survival in the different temperatures of 25 °C, 30 °C, 37 °C and 40 °C. The reason for choosing the temperature range of 25°C to 40°C is to check the ability of LAB to grow within the range of normal body temperature. Temperature is an important factor to classify LAB as probiotic, because if the LAB is not able to survive within the selected temperature range, then they will not able to survive in the human gut and exert their effects. In the present study, both selected LAB cultures showed growth in all tested temperatures. The optimum temperature for growth was found as 37 °C. The recorded CFU/ml are shown in figure 5. Similarly, Pundir *et al.* (2013) isolated lactic acid bacteria from fermented foods and found growth at 25 °C, 37 °C and 40°C. Isolated probiotic bacteria by Forhad *et al.* (2015) also showed optimal temperature for growth as 37°C. Chakraborty and Bhowal (2015) also reported that the Lactobacillus isolates were able to grow within 30 °C to 50 °C and the optimum temperature for maximum growth was 37 °C. Hossain *et al.* (2018) isolated *Bifidobacterium* species from human milk and infant feces which showed the ability to grow at 37 °C to 42 °C.

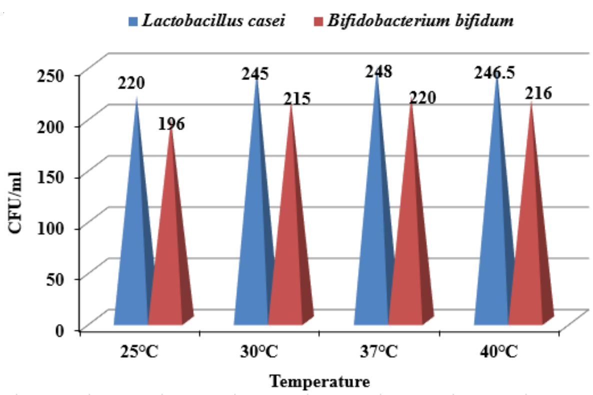

Fig. 5. Temperature sensitivity in selected LAB cultures

SUMMARY AND CONCLUSION

In the present study both the selectedLAB cultures (*Lactobacillus casei* and *Bifidobacterium bifidum*) were examined for their probiotic potential with parameters *i.e.,* different concentrations of bile salt, NaCl and phenol and range of pH and temperature. Both cultures showed growth in all tested concentrations of bile salt (0.5% to 2.0%) and phenol (0.1% to 0.4%) and temperature range (25 ºC to 40 ºC) whereas only 1% to 7% of NaCl concentrations showed growth of LAB cultures. *Lactobacillus casei* and *Bifidobacterium bifidum* showed growth in pH ranges of 3-8 and 3-7, respectively. The study concludes that both the selected cultures are good candidates as probiotics as these cultures showed the ability to tolerate the stressed conditions of the GI tract.

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Conflict of interest

The author declare no conflict of interests regarding the publication of this paper.

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