http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 660-663, 1999 Research Article

Inhibitory Effects of Probiotic Bacteria Against Selected Food-Borne Pathogens

Yazid, A.M[1], A.B. Salina[1], M. Shuhaimi[1], H. Osman[2] and J. Normah[1]
[1]Department of Food Technology, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[2]Department of Food Science and Nutrition,
Universiti Kebangsaan Malaysia, 47600, Bangi, Selangor, Malaysia

Abstract

Four probiotic microorganisms were tested for antimicrobial activity against selected food-borne pathogens namely *Escherichia coli*, *Salmonella enteritidis*, *Listetia monocytogenes* and *Bacillus cereus*. *Bifidobacterium breve* C11 and *Streptococcus faecalis* showed maximum antibacterial activity against all target microorganisms, while *Bifidobacterium infantis* C15 had the least antibacterial activity. Furthermore, the results of viable counts and pH profile revealed that the combination of probiotic microorganisms exerts better inhibitory effect against enteropathogenic *E. coli* than a single probiotic dose.

Introduction

The concept of probiotic was first introduced in early 1900's by Tissier (Fuller, 1989). However, the term was only used in 1965 by Lilly and Stillwell and has subsequently evolved. Numerous definitions have been proposed. Initially, Lilly and Stillwell (1965) defined probiotic as a substance secreted by a microorganism which can stimulate the growth of another microorganisms (Fuller, 1989). Today, a probiotic refers to any preparation "of live microorganisms which when applied to man or animal can beneficially affect the host by maintaining the intestinal microbial balance" (Havenaar and Huis, 1992). Lactic acid bacteria (LAB) namely lactobacilli, lactococci, streptococci, enterococci and *Bifidobacterium* spp. are the common microorganisms which have been used as probiotic preparations (Fuller, 1989).

The effect of probiotic organism on the micro ecology of the gut is to some extent dependent upon its ability to survive and preferably inhibit the proliferation of pathogens. In addition, various compounds produced during growth of the probiotic have been shown to inhibit the growth of pathogen such as *Vibrio cholera* and *Bacillus cereus* (Klaenhammer, 1988). These compounds include organic acids such as lactic and acetic acid, and antibiotic-like compounds such as reuterin and bacteriocin (Tagg *et al*., 1976). The organic acids lower the pH and thereby indirectly affect growth of the pathogen. Numerous bacteriocin have been reported to be produced by probiotic microorganisms namely Acidophilin, Bifidin and Nisin (Klaenhammer, 1988). They can either have a very broad range of activity or specifically inhibit the growth of very limited range of closely related microbes.

The objectives of the present investigation were to study the antagonistic action of probiotic microorganisms against selected food-borne pathogens and to elucidate the mechanism of such action.

Materials and Methods

Probiotic strains: The strains *Bifidobacterium breve* C11 and *Bifidobacterium infantis* C15 used in this study were obtained from the Probiotic Laboratory Culture Collection, Universiti Putra Malaysia (UPM). They were isolated from fresh infant stool. They were cultured in Trypticase Phytone Yeast-extract medium (TPY) as recommended by Scardovi (1986). *Bacillus mesentricus* TO-A and *Streptococcus faecalis* T-110 obtained from Toa Pharmaceutical, Japan and maintained propagated in nutrient medium (Lino *et al*., 1993) and Glucose-Yeast-Peptone (GYP) medium (Seo *et al*., 1989) respectively.

Target cultures of pathogenic organisms: The target cultures of *Escherichia coli*, *Salmonella enteritidis*, *Listeriz monocytogenes* and *Bacillus cereus* were obtained from the Tissue Culture Laboratory, UPM. They were maintained and propagated in Tryptone Soy (TS) medium.

Assay for inhibitory activity: Using a method modified from Vignolo *et al*. (1993), the 18 h culture of probiotic microorganisms were spotted on the respective agar medium using a sterile toothpick. *B. breve* C11, *B. infantis* C15 and *S. faecalis* T-110 were incubated anaerobically a 37°C for 48 h. *B. mesentritus* TO-A was incubated aerobically at 37°C for 48 h. The growing cultures were overlaid with soft (TS) agar seeded with 0.1 percent (v/v), freshly prepared target organisms. The overlaid cultures were incubated aerobically at 37°C for 24 h. The diameter of the inhibition zone was measured.

Inhibition of the growth of *Escherichia coli* in single and mixed cultures of probiotic microorganisms: Method used by Seo *et al*. (1989) was modified to study the inhibition of *Escherichia coli* by the probiotic isolates using the following combinations namely, combination (1)

S. faecalis T-110 against *E. coli*, (2) *B. breve* Cl 1 with *B. infantis* C15 against *E. coli*, (3) *B. breve* C11 with *B. infantis* C15 and *S. faecalis* T-110 against *E. coil* and (4) *B. breve* C11 with *B. infantis* C15, *S. faecalis* T-1 10 and *B. mesentricus* TO-A against *E. coli*. All the cultures used were at a concentration of 10^8 CFU/ml. After inoculation, the viable count of *E. coli* was determined at times intervals of 0, 2, 4, 6. 8, 10, 12, 16 and 24 h by plating it on McConkey agar and incubated at 37°C for 24 h aerobically. The pH of the mixed cultures was also measured at different time intervals.

Results and Discussion

B. breve C11 and *S. faecalls* T-110 showed maximum antibacterial activity followed by *B. mesentricus* TO-A and *B. infantis* C15 (Table 1). *B. mesentricus* TO-A showed maximum zone of inhibition against *B. cereus* and *L. monocytogenes* where it inhibited *B. cereus* greater than *L. monocytogenes*. The growth of *E. coli* and *S. enteritidis* were unaffected by the present of *B. infantis* C15 and *B. mesentricus* TO-A. *B. infantis* C15 showed the least antibacterial activity in most cases.

A combination of probiotic microorganisms was used for the treatment of diarrhea and constipation with enteropathogenic strains including pathogenic *E. coli* (Seo *et al.*, 1989). They reported that after 12-24 h *S. faecalis* and *C. butyricum*, either separately or combined, inhibited *E. coli* growth at a ratio of 1/100-1/1000. Yet a combination of *S. faecalis* and *C. butyricum* resulted a stronger effect and growth of *E. coli* was not observed on agar plates 36 h after inoculation with these strains. A symbiosis between these bacteria strongly inhibited the proliferation of *S. typhirnurium*, *V. parahaemolyticus*, *C. difficile* and *C. botulinum*.

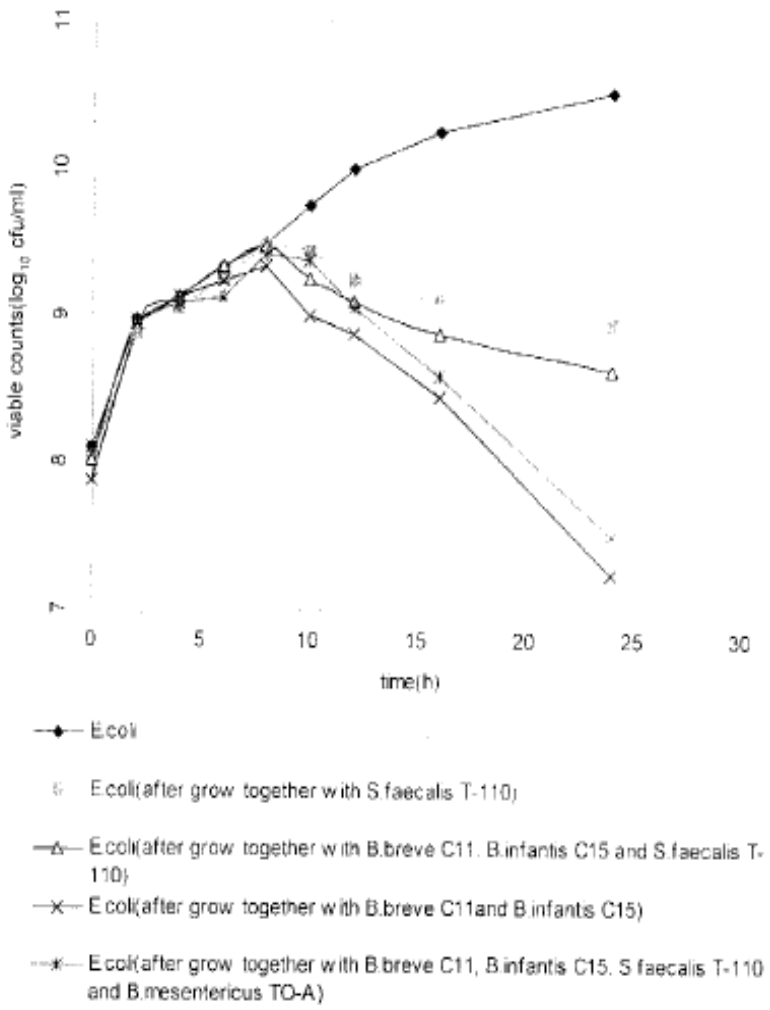

Fig. 1: Changes in counts of *E. coli* grown in pure and mixed cultures of probiotics

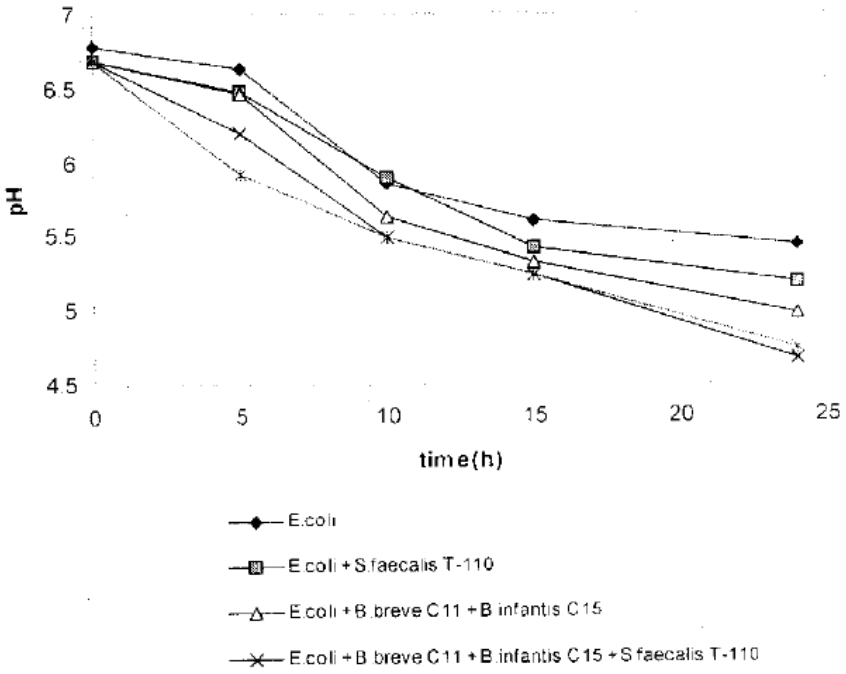

Fig. 2: Changes in pH of the culture medium

Table 1: Inhibitory activity of probiotic microorganisms against selected food-borne pathogens

Test organisms	Target organisms			
	E. coil	*B. cereus*	*S. enteritidis*	*L. monocytogenes*
B. breve C11	3.55	2.25	2.95	2.50
B. infantis C15	-	1.40	-	1.70
S. faecalis T-110	5.88	3.80	2.95	6.50
B. mesentricus TO-A	-	10.40	-	9.95

*Diameter in mm (without the diameter of the spot) of inhibition zone, -: Corresponds to the absence of clear inhibition zone

The results of the present study are shown in Fig. 1. The proliferation of combined probiotic microorganisms affected the growth of *E.coli* more significantly than did proliferation of a single probiotic. We found that after incubating *E. coil* with *S. faecalis* T-110 alone, the viable count of *E. coil* decreased to 10^1 CFU/ml after 24 h. However, when *E. coil* was inoculated with mixed cultures of *S. faecalis* T-1 10, *B. breve* 011 and *B. infantis* 015 simultaneously, the viable count of *E. coli* decreased to 10^2 CFU/ml. Likewise when *E. coli* was inoculated with mixed cultures of *B. breve* C11 and *B. infantis* C15, the growth of *E. coli* was inhibited and almost eliminated after 24 h. The decrease in viable count of *E. coli* was about 10^4 CFU/ml. The same pattern was observed when *E. coli* was grown together with *B. breve* C11, *B. infantis* C15, *S. faecalis* T-110 and *B. mesentricus* TO-A, where the decrease in viable counts of *E. coli* was about 10^3 CFU/ml after 24h. These findings show the establishment of the symbiotic relationship among probiotic microorganisms, which enhances the inhibition pathogenic *E. coli*.

The pH profile of the spent culture medium also suggested that the combination of probiotic microorganism significantly reduced the pH of the medium than a single probiotic did (Fig. 2). The medium pH of combination of *B. breve* C11, *B. infantis* 015 and *S. faecalis* T-110 against *E. coli* decreased by 2.12 percent (data not shown) companion with a pH of medium of *S. faecalis* 'T-110 alone which reduced by 0.06 percent (data not shown). This is due the fact that mixed *Bifidobacteria* and *S. faecalis* capable of producing much more acetic and lactic ac during their glucose metabolism (Scardovi, 1986). On contrary, the addition of *B. mesentricus* TO-A in the mi cultures of *B. breve* C11, *B. infantis* 015 and *S. faecalis* 110 slightly increased the medium pH by 0.04 percent (data not shown). *B. mesentricus* is known to produced amylase arid protease which are neutral and alka

respectively, a fact that might explain the increase in the pH of the medium (Leifert *et al*., 1995).

From the results discussed above, it clearly shows that the combination of the selected probiotic microorganisms exerts better inhibitory effect against food-borne pathogens than a single probiotic does. This antagonistic effect has been attributed much to the symbiotic association among the probiotic microorganisms themselves, which produce higher amount of bactericidal substances. Thus, they provide better prevention of disease to the host.

References

Fuller, R., 1989. Probiotics in man and animals. J. Applied Bacteriol., 66: 365-378.

Havenaar, R. and J.H. Huis, 1992. Probiotics: A General View. In: The Lactic Acid Bacteria in Health and Disease, Wood, B. (Ed.). Elsevier Applied Science, London, UK, pp: 209-224.

Klaenhammer, T.R., 1988. Bacteriocins of lactic acid bacteria. Biochimie, 70: 337-349.

Leifert, G., H. Li, S. Chidburee, S. Hampson and S. Workman *et al*., 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. J. Applied Bacteriol., 78: 97-108.

Lilly, D.M. and R.H. Stillwell, 1965. Probiotics: Growth-promoting factors produced by microorganisms. Science, 147: 747-748.

Lino, H., K. Fukaya, Y. Hirasawa, K. Shimizu and G. Seo, 1993. Stimulation of bacterial growth of some strains of Bifidobacterium by a crude preparation of metabolites from *Bacillus mesentericus* TO-A. Biomed. Lett., 48: 73-78.

Scardovi, V., 1986. Irregular Non-Spore Forming Gram-Positive Rods: Genus *Bifidobacterium* Orla-Jensen 1924. In: Bergey's Manual of Systematic Bacteriology, Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.). 9th Edn. Williams and Wilkins Publishers, Baltimore, MD., USA., pp: 1419-1435.

Seo, G.I., K. Shimizu, M. Sasatsu and M. Kono, 1989. Inhibition of growth of some enteropathogenic strains in mixed cultures of *Streptococcus faecalis* and *Clostridium butyricum*. Microbios. Lett., 40: 151-160.

Tagg, J.R., A.S. Dajani and L.W. Wannamaker, 1976. Bacteriocins of gram positive bacteria. Bacteriol. Rev., 40: 722-756.

Vignolo, G.M., F. Suriani, A.P.R Holgado and G. Oliver, 1993. Antibacterial activity of *Lactobacillus* strains isolated from dry fermented sausages. J. Applied Bacteriol., 75: 344-349.