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Antimicrobial effect of probiotic food against intestinal pathogens

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Abstract

A food can be defined as functional if it is shown to beneficially affect one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either the state of well-being and health, or to a reduction in disease incidence. The clinical situations studied presently include diarrhea, intestinal infections and colonization by pathogenic bacteria (including Helicobacter pylori and Clostridium difficile), so two different probiotic strains Pediococcus sp and Bifidobacterium sp. cointaining Dairy based probiotic drink were checked for their clinical benefits, which showed a good reduction in the number of intestinal pathogens. When the same experiment was conducted invitro the zone of inhibition clearly showed the antimicrobial effect of the probiotic strains against the intestinal pathogens. There are many mechanisms by which probiotics enhance intestinal health, including stimulation of immunity, competition for limited nutrients, inhibition of epithelial and mucosal adherence, inhibition of epithelial invasion and production of antimicrobial substances. From the findings it is clear that probiotic cultures are efficient in the removal of colonization of intestinal pathogens to the host intestine thereby giving protection to the host body.

Key words: Probiotics, Fermented foods, colonization, Pathogens, etc.,

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INTRODUCTION

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and or the activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). At the start of the 20th century, the term "Probiotics" was first introduced in 1953 by Werner Kollath (Hamilton-Miller *et al.,*2003). A food can be defined as functional if it is shown to beneficially affect one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either the state of well-being and health, or to a reduction in disease incidence (Salminen *et al.,* 1998). Probiotics were thought to beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin producing bacteria. Today, specific health effects are being investigated and documented including alleviation of chronic intestinal inflammatory diseases **(**Mach, 2006), prevention and treatment of pathogen-induced diarrhea (Yan and Polk, 2006) , urogenital infections, and atopic diseases. The word "probiotic" was derived from the Greek word which means "on be half of ". The concept was introduced by Lilly and Stillwell (1965) and was intended to stimulate substances produced by one microorganism to enhance the growth of another. Probiotic therefore is the exact opposite of antibiotic.The ability of lactobacilli and Bifidobacteria to survive in and colonize the gastrointestinal track has been associated with various health promoting properties (Ballongue, 2004). The colonization of probioticbacteria decreased with the increase of age of the host (Ballongue,2004). In recent years there has been interest in incorporating those bacteria in live form (called probiotics) into food especially fermented milk to counteract harmful bacteria in the gastrointestinal track and to promote health effect (Fuller, 1989 ; Schillinger *et al*., 2005, Tamime *et al.,* 2007). Several criteria have to be met for selecting probiotic strains those include acid and bile tolerance, survival through the gastrointestinal tract, ability to adhere to intestinal surfaces, exhibiting antimicrobial activity against potential pathogenic bacteria (Ouwehand *et al.,* 2004).

In the present work strains of *Pediococcus sp. and Bifidobacterium sp.* were isolated from various fermented cereals and evaluated for their potentiality to be used as a Probiotic. The isolated organisms were used to prepare Dairy based probiotic yoghurt. Their effect on removal of pathogenic microbes and its antimicrobial effect against food borne pathogens were detected.

MATERIALS AND METHODS

Effect on pathogenic microbes

One of the prime roles of probiotic organisms is providing immunity by competitive inhibition of pathogenic microorganisms especially coliforms from

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binding to the intestinal mucosa, and the inhibition of food borne pathogens (Midolo *et al*., 1995). In order to study the effect of probiotics in removing the pathogenic coliforms, fecal coliform count was done by spread plate method and antimicrobial sensitivity test was done to detect its role against food borne pathogens (Mack *et al*., 1999).

Fecal coliform count

Total microbes present in the trial animal feces were counted on weekly intervals by spread plate method by using Eosin methylene blue agar; the colonies formed were counted and recorded.

Antimicrobial activity against Food borne pathogens

Agar diffusion assay was used for the detection of antimicrobial activity of probiotic product against food borne pathogens. Muller-Hinton agar was prepared and inoculated with bacterial test pathogen such as *Escherichia coli, Salmonella enteritidis, Clostridium difficile* and *Helicobacter*. 50µl each of three different yoghurt samples (S2, S3 and S2S3) were added to wells punctured on the plate. They were then incubated at 37ÚC for 24 h. The diameter of the zone of inhibition was measured and recorded.

Table.2. Antagonistic activity due to probiotic product

RESULTS AND DISCUSSIONS

Effect on pathogenic microbes

Fecal coliform count

Probiotic organisms were efficient in the removal of pathogenic coliforms from intestinal mucosa, which was studied by spread plate method using Eosin methylene blue agar. There was a increase in the number of coliforms (Fig.1) removed at the end of every week. The difference in the fecal coliform count due to S2, S3 and S2 and S3 are perceived to be significant over control based on regression analysis (Table 1, 1a-c).

Table.1. Coliform count

S2 – *Pediococcus sp.* S3 – *Bifidobacterium sp.*, S2S3 – *Pediococcus sp* + *Bifidobacterium sp.*

Treatment	Number of weeks					
	1	2	3	4	5	6
Control	16	32	40	51	68	73
S2	22	39	43	57	75	87
S3	18	41	58	69	79	90
S2S3	19	39	48	61	78	87

Table.1a.

R	R Square	Adjusted R Square
0.999	0.998	0.997

Table.1b.

	Sum of Squares	df	Mean Square	F	Sig
Regression	2361.997	1	2362		0
Residual	5.337	4	1.334	1770.43	(S)
Total	2367.333	5			

S – Significant

Table.1.c.

	Unstandardized Coefficients		Standardized Coefficients	t	Sig
	B	Std. Error	Beta		
Constant	-0.95	1.226		-0.776	0.48
S2S3	0.861	0.02	0.999	42.08	0

Table.2. Antagonistic activity due to probiotic product

S. No.	Pathogenic organism	Test strains			
		S2	S3	S2S3	Control
1	*Clostridium difficile*	16mm	18mm	15mm	16mm
2	*Salmonella enteritidis*	22mm	23mm	24mm	23mm
3	*Escherichia coli*	15mm	17mm	19mm	16mm
4	*Helicobacter*	NA	NA	NA	NA

NA – No Activity

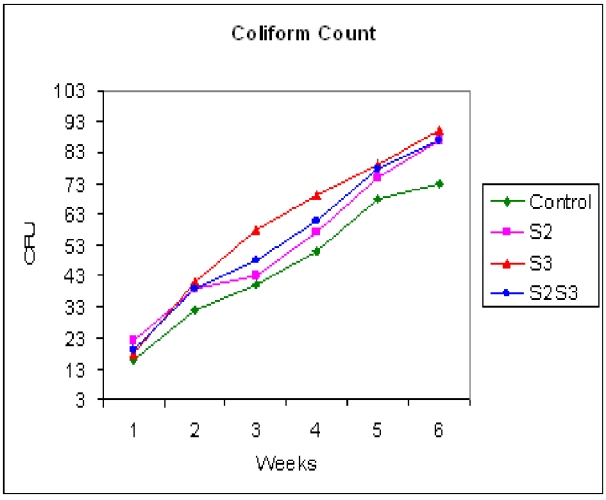

Fig.1. Coliform Count

It is very obvious that the number of organisms removed in the first week was less which could be due to the extension in the adaptability of the probiotic strains to the intestinal tract of the host. Similarly stress in the gastro intestinal region also reduces the number of probiotic organisms in the stomach, and hence the load of pathogens might be high. So it could be suspected that only very few probiotic organisms that can tolerate the stressful environment in the stomach were involved in the defense mechanism for the removal of the pathogens. However in the subsequent weeks they became very efficient in the removal of pathogens. This could be due to competition for attachment or adherence to the intestinal epithelium, and many reports suggest that the strains of *Bifidobacterium* are more efficient in adhesion to the intestinal mucosa.

In a similar study, Shi-Shun Zhong *et al.* (2004) reported that *Bifidobacteria*, the predominant bacteria, in the human intestinal microflora are considered to be a microorganism with a great influence on human health, and having inhibitory properties against enteropathogenic bacteria. Similarly Bernet *et al.* (1993) reported the occurrence of *Bifidobacteria* adhered to the human intestine by a mechanism of adhesion which involves a proteinaceous component.

Similar work was also done with *L. acidophilus* by Fourniat *et al.* (1986) and reported that the administration of killed *Lactobacillus acidophilus* in mice infected with a strain of Enterotoxigenic *E.coli* extended their survival. A protective effect of probiotic fermented food mixture was found in a similar model of mice infected with *E. coli* (Rani and Khetarpaul, 1998).

Antimicrobial activity

Probiotic organisms were antimicrobial in nature against food borne pathogens. The probiotic organisms (S2, S3, S2S3) were able to control the growth of *Clostridium difficile, Salmonella enteritidis* and *E. coli* and their effect is comparable to the antibiotic tetracycline. However these organisms did not have any effect on *Helicobacter* (Table 2).

Lactic acid bacteria exert strong antagonistic activity against many microorganisms including food spoilage organisms and pathogens by producing bacteriocins (Brinkten *et al.*, 1994). Several metabolic compounds produced during lactic acid fermentation (including organic acids, fatty acids, hydrogen peroxide and diacetyl) showed antimicrobial activity. However, bacteriocins are proteinaceous substances that have specific inhibitory activity against pathogens (Ouwehand,1998; Strus *et al.*, 2002; Xiao *et al.*, 2003)

Rodriguez (1996) reported that Nisin, a product of LAB showed inhibitory effect against a wide variety of Gram-positive food- borne pathogens and spoilage organisms, and also acted against several Gram negative bacteria by disrupting the integrity of their outer membranes (Kordel and Sahn, 1986; Stevens *et al.,* 1991). Nisin was also used to control the growth and spore formation of *Clostridium botulinum* and *Clostridium sporogenes* in cheese (Mattick and Hirsch, 1956). Thus probiotic organisms especially LAB can be used as a preservative as well as antimicrobial agent against human pathogens.

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