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Research Article

Antibacterial Activity of Probiotics Against Selected Human Pathogens

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Abstract

Probiotics are live bacteria that confer good health benefits to their host. Probiotics help in restoring the gut microbiota. A Probiotic bacterium inhibits the pathogen's growth and their attachment to the intestine or gastrointestinal tract. They help in the permeability of the intestine and increase the production of mucosa and intestinal integrity. In this study, it is an attempt to analyze the antibacterial effect of probiotic bacteria against common human pathogens. Eleven probiotic isolates were isolated from three different natural samples and they were characterized based on Colony Characteristics, Cell morphology, Staining properties and catalase test. The antibacterial activity of the probiotic isolates was determined against four human pathogens through the agar well diffusion method. Almost all the probiotic samples exhibited antibacterial activity against the tested bacteria.

Keywords: Probiotics; Gut Microbiota; Antibacterial Effect; Human Pathogens

Introduction

The gastrointestinal tract of a human adult is colonized by approximately 10^{14} microbial cells (Luckey and Floch 1972), 10 times more than that of our tissue cells [1]. The gut has gram-positive, anaerobic genera *Bacteroides*, *Bifidobacteria,* and *Eubacteria* are the dominant ones. Other than this, *Lactobacillus, Clostridia,* and *Streptococci* also play important role in the gut [2,3].

Gut microbiota includes various microorganisms like bacteria, fungi, archaea, viruses, and protozoans [4]. The gut microbiota should be maintained as they play an important role in the metabolism of nutrients, xenobiotics, and drugs. They also maintain the structural integrity of the gut mucosal barrier. They help in immunomodulation as well as protect against pathogens.

The gut microbiota is also playing a very important role in the functioning of the brain through bidirectional interactions between the nervous system and the gut. By interacting with the central nervous system, they help in the regulation of brain chemistry. They also influence various functions of neuro-endocrine systems like a response to stress and anxiety [5].

The gut microbiota has been altered due to infections in the gastrointestinal tract. Inflammatory bowel disease and irritable bowel syndrome can also make such changes [6]. To restore the gut microbiota, probiotics can be introduced into the gastrointestinal tract to enhance the functionality of existing microbial communities. Probiotics can restore gut microbiota and help in the prevention of gut inflammation and other diseases [7].

Vergio in 1954 for the first time introduced the term "Probiotics". He used the term Probiotika in his manuscript where he compared the effects of antibiotics and other antimicrobial substances on the gut microbiota. In 1965 Lilly and Stillwell referred to probiotics as "microorganisms promoting the growth of other microbes" [8]. Parker said in 1974 that probiotics are those "organisms that influence the gut microbiota and confer beneficial effects on host". In 1989, Fuller described probiotics as "live strains used as feed

for exerting beneficial effects for the host as well as improving the microbial balance in the intestine" [9]. Havenaar and Huis In't Veld in 1992 elaborated fullers definition and finally referred to probiotics as "mono or mixed culture of live strains of microbes taken by animal or human, that confer good health benefits to the host and also improve the gut microbiota" [10,11].

Probiotics are considered GRAS i.e. generally regarded as safe [12-14]. The mucosal membrane provides a surface for the microbiota to adhere there and confer health benefits to its host [15,16]. Many factors like nutrition, environment, and physiological and microbiological factors are also responsible for the influence of this microbiota on health [17-19].

Materials and Methods

Isolation and characterization of Probiotics from natural samples

Eleven probiotic isolates M1, M2, M3, CS1, CS2, CS3, CS4, V1, V2, V3 and V4 were isolated from various samples like Milk, Curd Samples, and Vagina respectively [20]. Characterization of all eleven isolates was carried out based on of their colony characteristics, cell morphology, Gram staining, Spore staining, Capsule staining, and Catalase test.

Bacterial pathogens

Four human pathogens *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98), *Shigella flexneri* (MTCC 1457), and *Staphylococcus aureus* (MTCC 3160) were procured from MTCC Chandigarh. These bacteria were stored on N-Agar slant (Hi-media) at 4.00 ± 2.00°C for further use.

Study of antibacterial activity of probiotics

Antimicrobial activity against selected pathogens was carried out by Kirby Bauer's diffusion method for CFC (cell-free culture/ supernatant) of probiotic isolates against different human pathogens and zone of inhibition and zone index were measured [20]. Activated cultures of probiotic isolates were inoculated in freshly prepared MRS broth and incubated at 37.00 ± 2.00°C for 24h. The Supernatant (CFC) was collected by centrifugation at 3000 rpm (1107g) for 15 min to study antibacterial activity against selected pathogens.

Result and Discussion

Antibacterial Activity of Probiotic Isolates					
		***Escherichia coli* (MTCC 443)**	***Shigella flexneri* (MTCC 1457)**	***Salmonella typhi* (MTCC 98)**	***Staphylococcus aureus* (MTCC 3160)**
1	M1	7	13	5	7
2	CS1	9	9	3	-
3	M2	7	10	6	6
4	M3	6	11	-	-
5	CS2	9	12	5	6
6	CS3	5	10	-	-
7	CS4	6	-	-	-
8	V1	8	12	7	6
9	V2	5	10	-	-
10	V3	10	9	-	6
11	V4	10	12	6	-

Table 1

Figure 1

Figure 2

All isolated Probiotic showed antibacterial activity against *Escherichia coli* (MTCC 443) *and Shigella flexneri* (MTCC 1457). The antibacterial activity of probiotic isolates was maximum against *Shigella flexneri* (MTCC 1457). Thus, further, probiotic isolates need to be confirmed by 16s rRNA sequencing and their antibacterial properties can be explored.

Conclusion

Eleven Probiotic bacteria were isolated from natural samples like Milk, Curd, and Vagina. Probiotic isolates were characterized based on colony characteristics, cell morphology, Catalase test, Gram staining, and Spore Staining. All the isolates were Gram-positive, non-spore formers, and catalase-negative. From the antibacterial activity, it was clear that all probiotic isolates showed a good range of inhibition towards the pathogens.

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