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ABSTRACT

Greek word probiotics "for life" are defined as 'live microorganism which beneficial effect on the health and well-being' of the hosts by maintaining the balance of gut micro flora. For the first time 'probiotic' term was used by Lilly and Stillwell in 1965 for those microorganisms by which substances secreted by them stimulate the growth of another. Elie Metchnikoff, the Russian zoologist and Nobel laureate is credited for first observing the important role of some bacteria in maintaining good gut microbiome who also postulated that due to the consumption of fermented milk containing lactobacilli, replaced the putrefactive bacteria in the gut results in healthy and long life of Bulgarian peasants. Globally there is a swift rise in the consumption of probiotics.

Probiotics are beneficial bacteria which alter the balance in gut microbiota, promote good digestion, inhibit the growth of harmful and pathogenic bacteria, also boost immune function and increase resistance against infection. Numerous functions exhibited by probiotics include production of vitamin, cholesterol lowering, alleviation of lactose intolerance, cancer prevention, stimulation of antibiotic associated diarrhea. Probiotics are used in human and animal feeds that include dairy and non-dairy products like cereals, baked foods, fermented meat products and dry foods.

To exert beneficial effects, probiotics should be able to maintain their viability in gastrointestinal tract (GIT) by surviving under harsh conditions like low p^H, acid, bile and pancreatin. GI microbiota is also influenced by various external and internal, host-related factors. External factors include the type of food eaten, microbial load of the immediate environment, feeding habits and the composition of the maternal microbiota. The successions of microbes are influenced by dietary and temperature-related stresses. Internal factor includes intestinal p^H, microbial interactions, environmental temperature, and physiological factors like peristalsis, bile acids, host secretions and immune responses (drug therapy and bacterial mucosal receptors). Other than delivery mode, infant diet, antibiotics, and other factors like host genetics, age, life style, hygiene factors, allergen contact, diets and consumption of pro- or prebiotics or infections might influence

the complexity of gut microbiota. The enhancement of the epithelial barrier, increase in the adhesion to intestinal mucosa (inhibit pathogen adhesion), competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances like bacteriocins and modulation in the immune system are the various mechanism by which probiotics exert beneficial effects. The antagonistic activity of LAB is due to the production of antimicrobial compounds such as lactic acid, ethanol, hydrogen peroxide (H_2O_2), diacetyl, reuterin, bacteriocin and biosurfactants. Due to a wide range of antimicrobial effects against many food borne pathogens, LAB widely used as natural preservatives, as bio-preservatives, extend shelf life which controlled food borne pathogens and has application in many industries and also used in various commercial purposes.

Lactic acid has a long history of uses for fermentation for preservation of fruits, vegetables and dairy products. For thousands of year diverse fermented food products have been prepared and consumed by humans who exert beneficial health effects on the hosts. The unique characteristic feature of lactic acid bacteria has attracted the scientific attention to explore their various applications in food as additives. These are also used in the production of poly lactic acid (PLA) which has great market potential into packaging, agriculture, automobile, electronics, textile and others industries The use of bacteriocins in bio-preservation systems meet consumers' demand for natural preservatives and also considered an additional safety measure to minimally processed products, which totally depends only on refrigeration as a conservation medium. Probiotics are reported to also have anti-mutagenic, anti-carcinogenic, hypo-cholesterolemic, anti-hypertensive, anti-osteoporosis and immune-modulator effects. Lactic acid bacteria (LAB) are regarded as Generally Recognized as Safe (GRAS) by WHO, plays an important role in the process of fermentation of food by inhibiting spoilage bacteria and production of flavour, aroma, and texture of fermented food.

Probiotic belong to heterogeneous group of lactic acid bacteria (LAB) *i.e Lactobacillus, Streptococcus, Leuconostoc, Enterococcus, Lactococcus, Pediococcus, Bifidobacterium*, and yeast *Saccharomyces boulardii*. Amongst all these members of the genera *Lactobacillus* and *Bifidobacterium* are the most frequently used probiotics due to

its uncountable benefits, while species of the genus *Streptococcus* and *Enterococcus* are now considered as opportunistic pathogens and are not generally preferred. These are non spore forming, Gram-positive with rod shaped, catalase and oxidase negative bacterium. Members of this genus are fastidious, they have complex nutritional requirements of amino acids, vitamins, nucleic acids and fermentable sugars for active growth. Lactic acid is the main product, produced as they ferment carbohydrates primarily glucose in the raw materials to produce various metabolites which give food its unique flavor and increase nutritional value of the fermented food.

The present study entitled " Isolation and Characterization of Lactic Acid Bacteria from Milk and milk Products and their Application in Preparation of Yoghurt'' was planned for the isolation of lactic acid bacteria from various milk and milk products and evaluation for their various beneficial properties to select those potential that could be used for the development of novel probiotics. The aim of our study included:-

1. To isolate, identify and purify different LAB from various milk and milk products.
2. To test their different characters of commercial importance like flavor, growth, production of useful products *etc.*
3. To find out the possibilities of commercial applications of selected LAB for production of high quality yoghurt.

This comprehensively documented the isolation and characterization of Lactic acid bacteria from various milk and milk products. The characterization of the isolates was performed for desired probiotic attributes, antimicrobial activity against various food borne pathogens and ability to produce various enzymes. In particular, strains G4, C28, C9, Cu20, P37, Cu12, Bu1 and B23 fulfilled the functional criteria required to be used as potential probiotics, amongst all the strains C9 showed the highest per cent probiotic attributes and highest antimicrobial spectrum against all the food borne pathogens. The summary of this study including significant findings and conclusions is presented as follows:

1. One hundred twenty samples of milk and milk products comprising cow milk, buffalo milk, goat milk, curd, paneer and buttermilk samples were collected from various districts of Meerut, Uttar Pradesh.

2. From these samples two hundred microbial cultures were isolated from these samples by using serial dilution method in Man Rogosa Sharpe (MRS) agar.

3. Pure culture by streak plate method were isolated from the above two hundred microbial isolates and preserved at -20°C in glycerol stock solution.

4. Out of two hundred isolates, one hundred fifty were identified as Gram positive of which one hundred twenty five bacteria were rods and coccobacilli and twenty five isolates were identified as yeast cultures. Fifty isolates appeared as pink rods or cocci which were identified as Gram negative cultures.

5. Endospore staining of one hundred twenty five Gram positive bacterial isolates suggests absence of endospore in one hundred five isolates, considered as endospore negative and twenty isolates were found as endospore positive.

6. All one hundred five endospore negative isolates were found to be catalase and oxidase negative.

7. Eight isolates namely G4, C28, C9, Cu20, P37, Cu12, Bu1 and B23 were shortlisted based on their per cent survival rate in all acid, bile and pancreatin tolerance test. All the isolates exhibit good tolerance results in acid, bile and pancreatin test.

8. In acid tolerance at p^H 3 after 3 h of incubation, the highest per cent survival rate was reported by G4 followed by Bu1, C9, C28, Cu12, Cu20, P37 and B23. Per cent survival rate after 6 h of incubation at p^H3 was recorded by G4 followed by C28, Cu20, C9, Cu12, P37, Bu1 and B23. At p^H 2.5 the highest per cent survival rate after 3 h was reported by C28 and Cu20 followed by G4,

P37, C9), Cu12, Bu1 and B23 whereas after 6 h of incubation all the eight isolates were able to survive.

9. In bile tolerance test, the highest per cent survival of isolates at 3 h incubation showed by Cu20, C28, G4, C9, P37, Cu12, B23 and Bu1 demonstrated only good tolerance. After 6h of incubation, the maximum per cent survival rate was exhibited by C9, Cu20, C28, G4, Cu12, P37, Bu1 and B23.

10. The highest test per cent concentration of pancreatin (0.5% w/v) at 3h incubation, G4, C9, C28, B23, Cu20, P37, Cu12 and Bu1 showed high to good tolerance for pancreatin. However with increase in time period of incubation up to 6h leads to the decrease in the per cent survival rate of the isolates which showed lesser tolerance but still survived, C9 the highest surviving isolates followed by C28, G4, P37, Cu20, Cu12, Bu1 and B23.

11. Isolates C9 exhibit highest auto-aggregation ability followed by C28, G4, Cu20, B23, P37, Cu12 and least by Bu1.

12. Amongst all the eight isolates, C9 exhibit highest cell surface hydrophobicity followed by C28, Cu12, B23, Bu1, P37, Cu20 and least by G4.

13. Isolate C9 exhibited antimicrobial activity against all the twelve food borne pathogens; G4, C28, Cu12 and Cu20 against eleven pathogens; P37 and B23 against ten pathogens; and Bu1 against nine pathogens.

14. Carbohydrate fermentation test for all eight isolates showed that all but B23 were xylose negative which fermented it. Except P37 rest seven isolates were lactose positive. G4 and C9 both were positive only for arabinose while Cu12 was positive for all 3 of the 12 sugars and was negative for arabinose, cellobiose, galactose, maltose, mannitol, ribose, sorbitol, sucrose and xylose. C28 showed positive fermentation test for 8 of the 12 sugars and was negative for arabinose, glucose, ribose and xylose. Bu1 was selectively positive for galactose, glucose, lactose and sorbitol and showed negative reaction against

rest of the 8 sugars. Cu20 was positive for 5 of the 12 test sugars which included cellobiose, galactose, lactose, mannitol and sucrose.

15. Out of eight isolates only four isolates namely G4, C9, Cu20 and B23 were able to hydrolyze arginine.

16. All the eight isolates exhibit a non- haemolytic activity.

17. Four isolates, namely G4, C9, Cu20 and Bu1 showed halo zones surrounding the colonies which indicate positive for the production of extracellular amylase enzyme.

18. Isolates G4, C28, Cu20 and Bu1 were lipase producers.

19. All the eight isolates namely G4, C28, C9, Cu20, P37, Cu12, Bu1 and B23 were found positive for the phytase production.

20. Only three strains, namely P37, Bu1 and B23 were protease positive.

21. All the eight isolates gave negative result in the production of gelatin.

22. All the eight isolates were susceptible to chloramphenicol. Isolate G4 was susceptible to nalidixic acid, gentamycin, erythromycin and intermediate to ampicillin, penicillin and streptomycin. C28 was susceptible to chloramphenicol, ampicillin, streptomycin and gentamycin whereas intermediate to penicillin, nalidixic acid and erythromycin. C9 was susceptible to penicillin, nalidixic acid and gentamycin. Isolate Cu20 was susceptible to all the antibiotics except ampicillin and pencillin. P37 was intermediate for nalidixic acid and gentamycin. Isolate Cu12 showed susceptibility to ampicillin, penicillin and erythromycin. Isolate Bu1 showed susceptibility against all the antibiotics except streptomycin and erythromycin. B23 exhibit susceptibility against all the antibiotics except nalidixic acid.

23. On the basis of the results it was established that the isolate namely C9 exhibited best performance in probiotic attribute tests and in respect of other desirable characterization. The isolate C9 belonged to the cow milk sample.

24. On the basis of 16S rRNA sequencing and BLAST analysis in GeneBank database of microbes resulted that isolate C9 was identified as *Lactobacillus fermentum,* shared 99% similarity. The partial 16S rRNA gene sequences of these strains were submitted to GenBank database under the accession no. MN421922 for C9 in NCBI (https://www.ncbi.nlm.nih.gov/nuccore/MN421922).

25. Yoghurt production with different fruit juices by C9 isolate accomplish in less than 3h. During incubation, the bacteria metabolize certain compounds in the milk which leads to flavor in yoghurt.

26. Sensory evaluation of yoghurt produced from different level of fruit pulp. Panelists liking yoghurt color, appearance, body and texture, flavor and over all acceptance was totally influenced by the concentration of fruits used in the yoghurt formulations. All the four different flavor yoghurt was liked by panelist mostly banana flavor followed by mango, papaya and apple.

27. Efficiency assessment of fingerlings in fish Rohu (*Labeo rohita*) was evaluated with C9 culture. Lowest feed conversion ratio was recorded for fingerlings fed with diet containing probiotic isolate C9 whereas fingerlings fed with control (lacking test probiotic cultures) showed highest feed conversion ratio. Highest mean length and mean weight, specific growth rate and condition factor was recorded in fingerlings fed with diet containing probiotic isolate C9.

In conclusion, the isolated and finally selected strains C9 (*Lactobacillus fermentum*) have a great probiotic value and can be safely used as starter culture, food additives and in nutraceuticals for their possible good advantages and health benefits for the host. Finally