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Research Paper

A STUDY ON THE PROBIOTIC ASPECTS OF LACTOBACILLUS ISOLATED FROM RAW MILK OF VECHUR

Director publisher

ABSTRACT

The objective of this study was to characterize the probiotic attributes of a Lactobacillus species isolated from raw milk of Vechur, a local indigenous cow breed of Kerala.

Lactobacillus was isolated by selective enrichment of milk samples in MRS broth and subsequent plating in MRS agar. The results of biochemical characterization were suggestive of the isolate to be L.acidophilus. Considerable growth observed even after 3h of exposure to pH 3.0 and three percentage of bile salt concentration confirmed the ability of isolate to withstand the harsh environment of gastrointestinal tract. Opaque white colonies on Lactobacillus Oxgall agar affirmed the BSH activity of the isolate.CSH values obtained with the different solvents: n-hexadecane, ethyl acetate and chloroform were 36%, 30% and 27% respectively. The aggregation percentage of 52% in 24h indicates the good adhesion potential. Absence of resistance to tetracycline can be taken as an indicator for the absence of acquired antibiotic resistance. Observations made in this trial suggest that the isolate used in this work has ample potential to be exploited as a probiotic starter.

KEYWORDS :

INTRODUCTION

Lactic acid bacteria are integral components of fermented foods where they carry out primary and secondary fermentation. Their long history of safe use in foods have earned them the status Generally Regarded As Safe (GRAS).Lactic acid bacteria consti- tute a group of gram positive bacteria united by a constellation of unique morphologic metabolic and physiological characteris- tics. They are the major starters used in the preparation of fer- mented milk products. Lactobacillus, one of the most important genus of lactic acid bacteria comprise of a large diverse group of gram positive, non sporing, catalase negative, rods that produce lactic acid as the major end product on fermentation of carbo- hydrates. Lactobacilli are widely and safely being used as probi- otics for medical and veterinary applications. However over the world, search for novel indigenous probiotic strains is continu- ing to satisfy the ever increasing demand of the market for novel strains to develop new functional products. Foodborne lactoba- cilli consist of natural, uncharacterized strains, whose biodiver- sity depends on geographical origin, seasonality, animal feeding/ plant growth conditions. The present study is an attempt for the probiotic characterization of a Lactobacillus species isolated from raw milk of indigenous cattle breed Vechur.

Materials and Methods

Raw milk samples were collected aseptically from the Vechur cows of the University Livestock farm, Kerala Veterinary and Animal Sciences University, Mannuthy. Isolation of Lactobacil- lus from milk was achieved by selective enrichment in MRS broth and subsequent plating in MRS agar as per the standard procedure. Subsurface colonies of Gram positive rods that were catalase and oxidase negative were selected, and maintained in nutrient agar slants at 4°C. Biochemical characterization of the isolate was carried out (Barrow and Feltham, 1993). For long term storage, isolate was also preserved in 70% glycerol at -20°C.

To ascertain the capacity of the isolate to withstand the hostile environment of acidity and bile in the human GI tract ,acid and bile tolerance of the isolate was assessed by judging the nature of growth (slight/ moderate/ heavy) obtained on streaking the culture in MRS agar after 3h exposure to pH 3.0 and 3.0 % bile salt concentration.

Ability of the isolate to produce bile salt hydrolase (BSH) was assessed qualitatively by comparing the colony morphology in MRS agar and bile salt supplemented MRS agar (Dashkevicz and Feighner, 1989).

Adhesion potential of the isolate was measured in terms of cell surface hydrophobicity (CSH) by Microbial Adhesion to Solvents (MATS) assay using the apolar solvent n- Hexadecane, acidic solvent chloroform and basic solvent ethyl acetate (Rosenberg *et al*, 1980).Affinity to hydrocarbons was reported as adhesion percentage as per the formula (A0-A/A0) ×100 where A0 and A werethe absorbance before and after extraction with organic solventsrespectively. Hydrophobicity was calculated from three replicates as the percentage decrease in the optical density of the original bacterial suspension due to cells partitioning into a hydrocarbon layer.

Auto aggregation ability a property related to adhesion was also determined as procedure of Reniero *et al,* (1992). The standard- ized broth suspension was incubated in aliquots at 37^0C and was monitored for 24h in terms of absorbance (A) at 600nm.Auto aggregation percentage was calculated by using the formula (1-A upper suspension/A total bacterial suspension) ×100. Autoaggre- gation was calculated from three replicates as the percentage de- crease in absorbance of the original suspension due to aggrega- tion and sedimentation. Sensitivity of the isolate to tetracycline, the most wide spread antibiotic resistant determinant wasdeter- mined by disc diffusion assay. (Bauer *et al*1966).

Results and Conclusions

Sub surface colonies obtained in MRS agar was indicative of the microaerophilic nature of the isolate. Microscopic examination of stained smear revealed the presence of Gram positive long ba- cilli arranged singly and in pairs. Preliminary identification tests and biochemical characterization results were suggestive of the isolate to be *Lactobacillus acidophilus*.

Bacterial adhesion to intestinal epithelial cells is important for colonization of probiotic strains in the gastrointestinal tract as it prevents their immediate elimination by peristalsis. This also provides a competitive advantage for the isolate in this ecosys- tem. Adhesion potential, a property primarily dependent on physicochemical characteristics of cell surface was determined *invitro* in terms of CSH and auto aggregation. According to Del Re *et al*, (2000), strains should possess a hydrophobic surface for better adhesion to intestinal cells. The bacterial adhesion to apolar solvent reflects the hydrophobicity of the cells. Maximum CSH value (36%) with the apolar solvent n-hexadecane suggests a hydrophobic cell surface for the isolate. As the difference in CSH values obtained for acidic solvent (27%) and basic solvent (30%) is very less, the results are neither indicative of a strong

acidic nor a strong basic character for cell wall.

Ability to auto aggregate is an advantageous property for probi- otic strains as it complements the adhesion potential. The auto aggregation percentage obtained for the *Lactobacillus acidophilus* isolate from Vechur cow milk was 52% within 24h. Similar values have been reported for *Lactobacillus acidophilus* NCFM (Collado *et al,* 2008). The CSH values and percentage of auto ag- gregation observed is very much supportive of the good adhe- sion potential of the isolate obtained in this work.

In recent years, ability of probiotic lactobacilli to produce bile salt hydrolase (BSH) has become the focus of attention, on ac- count of its influence on cholesterol metabolism. To assess the ability of the isolate to hydrolyze bile salt, the colony morpholo- gy in MRS agar with 0.3% bile salt was compared to that on sim- ple MRS agar (control). In the control plate, colonies were pale and translucent whereas in MRS agar with 0.3% bile salt, colo- nies were opaque and white due to the precipitation of bile ac- ids by way of bile salt hydrolase activity of the isolate. Distinctly different colony morphology in control plate and treatment plate was suggestive of BSH activity of the isolate. Probiotics en- counter significant amount of bile salts consistently in the mam- malian gut. As BSH helps in the detoxification of bile salts, pres- ence of this enzyme definitely improves the survival rate in the competitive environment of gastrointestinal tract (Fuller, 1989). The remarkable bile tolerance shown by the isolate used in this work could be due to BSH activity. Recently there has been an increasing interest in bile salt hydrolytic activity of lactic acid bacteria as they are being identified as biological hypocholeste- raemic agents (Ramasamy *et al*, 2010).

The most wide spread antibiotic resistant determinant in Lacto- bacilli is tet (M) Nawaz et al (2011). Absence of acquired antimi- crobial resistance is a prerequisite for the safety of lactobacilli to be used as probiotic starter. As the antibiogram revealed sensi- tivity to tetracycline, demonstrating the absence of acquired an- tibiotic resistance, it can be inferred that the isolate obtained in this work is a safe probiotic candidate.

Table. 1
Percentage of cell surface hydrophobicity
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	Sulphadiazine	R
	Amoxicillin	R
	Ampicillin	R
	Oxacillin	S
	Ciprofloxacin	S
	Clindamycin	S
	Chloramphenicol	S
	Ceftazidime	I
	Chloramphenicol	I
	Rifampicin	I

R- Resistant, S- Sensitive, I- Intermediate

Figure 1
Gram's staining of *Lactobacillus acidophilus*
ACKNOWLEDGEMENT:
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Sl. No	Solvent	CSH
1	n- Hexadecane	36%
2	Chloroform	27%
3	Ethyl acetate	30%

Table .2
Antibiogram of isolate

Sl. No	Name of the antibiotic	Result
	Cefixime	R
	Gentamycin	S
	Tetracycline	S
	Aztreonam	S

CONCLUSION

The most wide spread antibiotic resistant determinant in Lacto- bacilli is tet (M) Nawaz et al (2011). Absence of acquired antimi- crobial resistance is a prerequisite for the safety of lactobacilli to be used as probiotic starter. As the antibiogram revealed sensi- tivity to tetracycline, demonstrating the absence of acquired an- tibiotic resistance, it can be inferred that the isolate obtained in this work is a safe probiotic candidate.

ref_str

1. 1. Barrow G H and Feltham R K A (1993). Cowan and Steel's Manual for Identification of Medical Bacteria. Third edition. Cambridge University

Press, Cambridge. pp. 331. 2. Bauer AW, Kirby MM, Sherris J C and Truck M (1966).Antibioticsusceptibility testing by a standardizedsingle disk

method. Am J ClinPathol.,45:493-496. 3. Collado M C, Meriluoto J, Salminen S (2008). Adhesion and aggregation properties of probioticand pathogen strains. Eur Food Res Technol.,

226:1065–1073. 4. Dashkevicz M P and Feighner S D (1989) Development of a differential medium for bile salt hydrolase-active Lactobacillus spp. Appl Environ Microbiol. 1989 Jan;

55(1): 11–16. 5. Del Re B, Sgorbati B, Miglioli M and Palenzona D (2000).Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacterium longum. Lett Appl Microbiol.,

31(6):438-42. 6. Nawaz M., Wang J., Zhou A., Ma C., Wu X., Moore J. E., et al. (2011). Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr. Microbiol. 62, 1081–1089 7. Fuller, 1989 A Review: Probiotics in man and animals J. Appl. Bacteriol. 66: 365-378 8. Ramasamy, K., Abdullah, N.,Wong, M.C.V.L., Karu- than, C. & Ho, Y.W. (2010). "Bile salt deconjugation and cholesterol removal from media by Lactobacillus strains used as probiotics in chickens." J Sci Food Agric 90:65–69. 9. Reniero R, Cocconcelli P S, Bottazzi V and Morelli L (1992). High frequency of conjugation in Lactobacillus mediated by an aggregation-promoting factor. J. Gen. Microbiol., 138: 763-768. 10. Rosenberg M, Gutnick D and Rosenberg E (1980). FEMS Microbiol Lett., 9:29-33.

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