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HYPOCHOLESTERAEMIC POTENTIAL OF A *Lactobacillus* species ISOLATED FROM HOUSE-HOLD CURD

KEYWORDS: House hold curd, cholesterol assimilation, bile salt hydrolase

Dr. Beena A K	Anupa A	Twinkle J K
Associate Professor, Dept. Of Dairy Microbiology, Collegeof Dairy Science and Technology, Mannuthy, Mob:- 9446293686	Research Assistant, Dept. Of Dairy Microbiology, College of Dairy Science and Technology , Mannuthy, Mob:- 7736055647	Research Assistant, Dept. Of Dairy Microbiology, College of Dairy Science and Technology , Mannuthy, Mob:- 9946881648

ABSTRACT *Probiotics, which are known to bring about their beneficial effects by promoting intestinal integrity, are increasingly being used in nutraceuticals and functional foods. With cardiovascular diseases, contributing to a major proportion of the lifestyle diseases, probiotics targeting cardiovascular health are expected to be of great demand in the coming years. Most of the probiotic strains currently available in our market are of non-indigenous origins. However, the effectiveness of probiotic microorganism is often population specific due to the variations in food habits, gut microflora and host microbial interactions. Hence there is a strong need for exploring the probiotic potential of indigenous microbes. In this work, lactic acid bacteria isolated from a house- hold curd sample was biochemically characterized, identified and assessed for their cholesterol assimilation potential and Bile salt hydrolase (BSH) activity in vitro. Characterization results were suggestive of the isolate to be Lactobacillus acidophilus. The isolate was found to possess remarkable BSH activity and assimilate 47% cholesterol within 24h. The findings obtained in this work indicate that there is ample scope for exploring the other probiotic attributes of the isolate.*

Introduction

The risk of heart attack is three times higher in those with hypercholesterolemia when compared to those who have normal lipid profiles. Recent modalities for lowering blood cholesterol level include dietary management, behavior modification, regular exercise and drug therapy. Though low fat diet is very effective in reducing blood cholesterol level on a population basis, expected result is seldom attained due to the poor compliance that are often attributed to the low palatability and acceptability of these diets. So it is worth exploring the possibility of supplementing the daily diet with more acceptable and appealing products like fermented dairy products that contain beneficial lactic acid bacteria. According to the World Health Organization (WHO), cardiovascular disease will be the leading cause of death by 2030. Moreover todays' health conscious population prefers non pharmacological alternatives rather than drugs for reducing the risks associated with lifestyle diseases. In this context probiotics with its proclaimed health benefits become very relevant.

Lactic acid bacteria which are closely associated with human environment are also involved in large number of spontaneous food fermentations. Nature with its rich biodiversity is a treasure house of undiscovered wealth of molecular diversity. There are reports of wild strains of Lactobacillus being isolated from human associated environment. Cocktails of various microorganisms especially lactic acid bacteria belonging to lactobacillus are traditionally being used in fermented dairy products to promote human health.Lactobacilli are frequently used as probiotics in cultured milks, infant foods and various pharmaceutical preparations. Recently, WHO has identified the deconjugation potential as one of the desirable characteristics for probiotic microbes (Araya et al2002).Apart from improving the colonization potential, bile salt hydrolase activity also supplements the cholesterol lowering potential of the isolate. Considering the above facts, an effort was made to isolate *lactobacillus* species from an indigenous source and assess its BSH activity and cholesterol assimilation potential.

Materials and methods

Isolation of Lactobacillus was achieved as per the standard procedure of Harrigan, (1998). Ten gram of curd sample was transferred to 50ml of MRS broth and incubated at 37°C for 24 h for selective enrichment of Lactobacillus species. Appropriately diluted sample from this was pour plated using MRS agar and incubated at 37°C for 48 h so as to get well isolated distinct colonies. 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 preserved in 70% glycerol at -20°C.

Possession of bile salt hydrolase is known to improve the colonization potential, an important advantageous property for probiotics; as such bacteria have a better chance of survival in environment rich in bile salt. Moreover such strains are also expected to have an impact on lipid metabolism. So the ability of the isolate to hydrolyse bile salt was assessed qualitatively using Lactobacillus Oxgall agar bile at a level of 0.15 per cent. (Dashkevicz and Feighner, 1989) Opaque white colonies without precipitate halos and colonies with precipitate halos and were taken as positive for BSH.

Ability of the isolate to assimilate cholesterol was determined as detailed by Lye et al (2010). Freshly activated culture in MRS broth was used for the experiment. Filter sterilized water soluble cholesterol at a level of 10µg/ml was added to MRS broth supplemented with 0.15% L-cysteine hydrochloride.Media pH was adjusted to 8.0 to mimic the condition of small intestine.Freshly activated culture (10^8cfu/ml) was added to MRS broth containing cholesterol at a level of one percent. Incubation was done at 37°C for 24h. After removal of the cell pellet by refrigerated centrifugation, cholesterol content of the cell free broth was again determined (Ruddel & Morris, 1973). Amount of cholesterol assimilated by the isolate was calculated in percentage by finding the difference in the cholesterol con-

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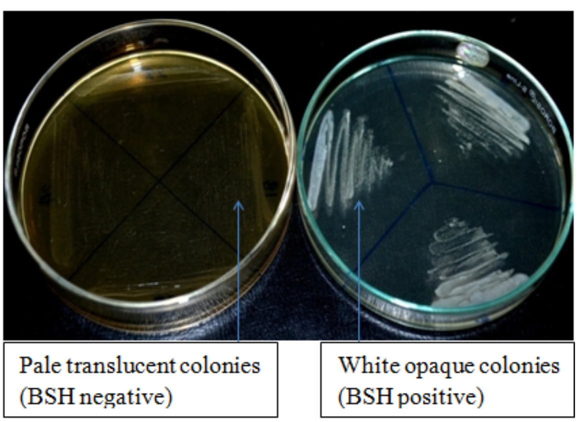
centration of MRS broth prior to and after fermentation. (Concentration of cholesterol was obtained from the standard curve with concentration of cholesterol on X axis and corresponding absorbance at 550nm on Y axis).

Result and Discussion

The isolate grew on the MRS agar as subsurface flat spindle shaped colonies. Sub surface colonies are indicative of the microaerophilic nature of the isolate (fig.1). Microscopic examination of stained smear revealed the presence of Gram positive long bacilli arranged singly and in pairs. Preliminary identification tests and biochemical characterization results were suggestive of the isolate to be Lactobacillus acidophilus (table.1). Probiotics are live microorganisms that beneficially affect the host by improving the intestinal microbial balance. For this, ability to withstand the hostile environment of bile and acid is essential. Lactobacillus is one of the genuses that are most widely recognized for its probiotic properties. Lactobacilli are often considered to be commensal or beneficial participants in human microbial ecology. There are numerous reports of L. acidophilus being isolated from the gut of humans (Pereira and Gibson, 2002), swine (Ahnet al, 2003) and chicken (Ramasamy et al, 2010). Being a constituent of normal flora of gut, it can be assumed that L.acidophilus has sufficient acid and bile tolerance to remain viable in gastrointestinal tract to confer the health benefits. Remarkable acid and bile tolerance has been reported for different strains of L.acidophilus (Dixit et al, 2013).

Fig.1 Bile salt hydrolase activity of Lactobacillus isolate



In recent years, ability of probiotic lactobacilli to produce BSH has become the focus of attention on account of its influence on cholesterol metabolism. To assess the ability of the isolate to hydrolyze bile salt, the colony morphology in MRS agar with 0.3% bile salt was compared to that on simple MRS agar (control). In the control plate, colonies were pale and translucent whereas in MRS agar with 0.3% bile salt, colonies were opaque and white due to the precipitation of bile acids by way of bile salt hydrolase activity of the isolate. Distinctly different colony morphology in control plate and treatment plate is suggestive of BSH activity of the isolate. Ahnet al (2003) reported precipitated halo and opaque granular white material for Lactobacillus acidophilus colonies capable of deconjugating bile salts. Deconjugation is catalyzed by bile salt hydrolase which hydrolyse the amide bond and liberate deconjugated bile acids glycine/taurine moiety from the steroid core (Bortolinni et al, 1997). Probiotics encounter significant amount of bile salts consistently in the mammalian gut. Overall data strongly support the hypothesis that microbial BSH helps in the detoxification of bile salts. The bile salt hydrolysis activity benefits the bacterium by enhancing its resistance to conjugated bile salts which in turn increase their survival rate and colonization (Jones et al2008). However there are reports of BSH negative strains of Lactobacilli capable of growing in media containing bile suggesting that bile tolerance is not necessarily exclusively the outcome of BSH production.

Table. 1 Biochemical characteristics of Lactobacillus isolate

PROPERTIES	RESULT
Catalase	-
Gas from glucose	-
Arabinose	-
Galactose	+
Lactose	+
Maltose	+
Mannitol	-
Melibiose	-
Raffinose	-
Salicin	-
Sorbitol	+
Sucrose	-
Trehalose	-
Oxidase	-
Indole	-
Methyl Red	+
VP	-
Nitrate reduction	+

In the cholesterol assimilation studies, it was found that within 24h, the isolate reduced cholesterol concentration in the growth medium by 47per cent (average of triplicate observations).The percentage of cholesterol removed by different strains of L.acidophilus ranged from 20- 57%, 43% - 71% and 11-52% when grown in MRS broth containing oxgall, cholic acid, and taurocholic acid respectively (Lin and Chen,2000).From this it can be inferred that the growth conditions of the organism do have a great influence on the cholesterol assimilation potential of the isolate. Ziarno M (2008) reported that percentage of cholesterol assimilation of different strains of L.acidophilus ranged from 8.4 to 16.1 in MRS broth when incubation was done for 5h.Walker and Gilliland (1989) opined that reduction of cholesterol in broth could be due to the precipitation of cholesterol with deconjugated bile salts. According to Brashears et al, 1998 removal of cholesterol from media can happen either by the incorporation of cholesterol on to the cell membrane or due to the destabilization of cholesterol micelle and its co-precipitation with deconjugated bile salts.

Bile salt hydrolysis has been reported for many Lactobacillus species isolated from fermented dairy products (Tanaka et al 2007). In this study also the isolate from household curd showed remarkable BSH activity. When deconjugated, rate of excretion of bile acids increases. Cholesterol being a precursor of bile acids, converts its molecules to bile acids, to replace those lost during excretion, such that serum cholesterol level is reduced (Smet et al, 1994). However, the level of deconjugation attainable with Lactobacillus species for various bile

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salts are very much strain dependent (Ramasamy et al, 2009). Recently there has been an increasing interest in bile salt hydrolytic activity of lactic acid bacteria as they are being identified as biological hypocholesteraemic agents. (Nguyen et al, 2007). Ramasamy et al (2010) reported that strains with good deconjugating ability need not always remove cholesterol efficiently. The isolate used in this work showed remarkable BSH activity as well as good cholesterol assimilation potential. The potential for bile salt hydrolysis is expected to complement the hypocholesteraemic potential of the isolate as deconjugated bile salts are less efficient in solubilization of lipids in the gut. Indigenous probiotic Lactobacillus strains are likely to have competitive advantage as they will be better adapted to the Indian population due to the local conditioning effect.

Conclusion

Recently there has been considerable interest in the effect of probiotics on lipid metabolism. The results obtained in this work are clearly suggestive of hypocholesteraemic potential of the isolate. The findings support the fact that the isolate obtained in this work has ample potential, for being used in probiotics especially those targeting cardiovascular health. As a means to tolerate the toxicity generated by conjugated bile acids, enteric strains are reported to hydrolyse bile acid amino conjugate into free bile acids which are excreted through faeces. Secondary bile acid production is considered as a significant risk factor for colon cancer. Hence studies to elucidate the fate of end products of bile salt hydrolysis are of vital importance. Though there are concerns over the safety of using BSH positive strains, it is not relevant for Lactobacillus species which are incapable of dehydroxylating deconjugated bile salts to secondary bile acids. With the BSH hypothesis being proposed for the hypocholesteraemic effect of many lactic acid bacteria, it is important to unravel the physiological impact of bile salt hydrolase activity on mammalian cells.

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