Preliminary Studies on Probiotic Properties of Bacterial Isolates from Natural Food Samples

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ABSTRACT:

The objective of this study was to characterize probiotic bacteria isolated from natural food samples viz. curd, cucumber and soy milk, focusing on their safety, antimicrobial, and probiotic properties. A total of seven isolates of bacillus was selected from 12 species of bacteria isolated from natural sources and were screened to study their probiotic properties. From all of the cultures 7 isolates of lactobacilli were selected for acid and bile tolerance, pH tolerance and NaCl tolerance. Almost all of the acid and bile tolerant isolates of lactobacilli were also showing antimicrobial properties. None of the assayed strains showed hemolytic, proteolytic or lipolytic activity indicating their status as safe cultures for formulating probiotic foods.

KEYWORDS:

Probiotics, Curd, Cucumber, Soy milk.

INTRODUCTION

Intensive research reports in recent era confirm the major importance of the microbial population of the gastro-intestinal tract (GIT). Probiotics are live microorganisms (in most cases, bacteria) that are similar to normal flora found in the human gut. They are also called "friendly bacteria" or "good bacteria." Probiotics are available to consumers mainly in the form of dietary supplements and foods. They can be used as complementary and alternative medicine (CAM). There is limited evidence supporting some uses of probiotics. Much more scientific knowledge is needed about probiotics, including about their safety and appropriate use. Effects found from one species or strain of probiotics does not necessarily hold true for others, or even for different preparations of the same species or strain. Probiotics are available in foods and dietary supplements and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and unfermented milk, miso, tempeh, and some juices and soy beverages. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation.

Probiotics are beneficial bacteria in that they favorably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection [1]. Other physiological benefits of probiotics include removal of carcinogens, lowering of cholesterol, immune stimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance [2].

In order to exert their beneficial effect, probiotics must survive in the gastrointestinal (GI) tract, persist in the host, and prove safety for consumer [3]. To survive in the gut, the organisms must be tolerant to low pH and bile toxicity prevalent in the upper digestive tract. However the culture should also possess properties to fulfill probiotic criteria of non proteolytic, non-lipolytic and non-haemolytic activities apart from other properties as mentioned in review of literatures in the current studies and prospects of Probiotics.

MATERIALS AND METHODS

Sample collection and Media preparation:

Isolation carried out from soy, cucumber and curd sample as well as from milk sample. Bacteria were isolated by using enrichment techniques in MRS broth. Nutrient agar, nutrient broth, de Man, Rogosa and Sharpe (MRS) agar and de Man, Rogosa and Sharpe (MRS) broth, used for bacterial growth were prepared according to the methods recommended by Harrigan and McCance [4]; and Harrigan [5]. The pH of media was adjusted by using 0.1N NaOH and 0.1N HCl. MRS Agar was prepared by adding 1.5% agar in MRS broth (prepared above) and was then autoclaved.

Inoculation and incubation:

The samples inoculated into MRS broth and incubated at 37° C for 48 hours than transfer subsequently to MRS agar for isolation and purification. The samples were stored under refrigeration conditions for subsequent experiments.

Cultural Characterization of Isolates:

The typical colonies on MRS agar were sub-cultured on the same medium again and again until a pure growth for each culture isolate was obtained**.** Morphological examinations to characterize the isolates on the basis of cell arrangement as well as cell size and shape were performed by Gram staining and by monochrome staining as well.

Biochemical Characterization of Isolates:

All the biochemical tests (As showed in Table 2) performed according to Bergey's Manual, media were inoculated with respective test organisms and incubated at 37 °C for 24 hours and results were subsequently observed.

Proteolytic Activity

In order to determine the proteolytic activities of strains, the identified isolates were cultivated on reconstructed agar plates (10%) containing skim milk medium and were incubated at 30°C for 18-20 hours. Colonies having a transparent ring around them were considered as strains indicating proteolytic activity [6].

Lipolytic Activity

To determine the lipolytic activities of strains, identified isolates were cultivated on medium plate containing Nutrient agar and 1% (v/v) & cream of milk (38% fat) and incubated at 37°C for 72 hours. Colonies having a transparent ring around them were considered as strains indicating lipolytic activity [7].

Haemolysis Activity

Haemolysis activity of gelatinase negative isolates was investigated as described by Gerhardt [8]. 2µl of a 6 hour old culture broth was spot inoculated into sterile blood agar. The blood agar was prepared by adding 10% human-blood sample

which was procured from pathological laboratory. Blood so collected was preserved in ethylene diamine tetra acetic acid (EDTA), into sterile blood agar base at 45°C. Plates were incubated anaerobically at 37°C for 48 hours after which they were observed for clear zones surrounding colonies (positive reaction for beta haemolysis). A strain of *S. aureus* was used as positive control. The test was carried out to check whether the culture isolate proves to be nonpathogenic or not which could serve as the criteria for selecting a probiotic culture.

Determination of Probiotic Properties:

Determination of Acid Tolerance

Percent survival of the three strains was determined after exposure to pH 2.5 for 2 hours and 4 hours at 37°C. For this, overnight grown cultures were inoculated in MRS broth adjusted to pH 2.5. The samples were plated onto MRS agar at the end of exposure time. The plates were incubated for 24 hours at 37°C and total viable count was determined. Broth without inoculation served as negative control while set of test organisms inoculated in MRS broth (pH 6.2) was used as test.

Bile Salt Tolerance

MRS Broth with 1%, 2%, 3%, 4% Bile salt (w/v) were inoculated with a 0.1ml activated culture of each bacterial suspension viz. PCd1, PCd3, PCd4, PCu1, PCu3, PS1, PS2 and incubated at 37°C for 48 hours. Growth of the organism was measured using spectrophotometer. The control comprised of MRS broth without bile salt. Bacterial growth was monitored by measuring absorbance at 600 nm after incubation for 24 hours at 37°C.

NaCl Tolerance

MRS Broth with 1%, 2%, 3%, 4% NaCl (w/v) were inoculated with a 0.1ml activated culture of each bacterial suspension viz. PCd1, PCd3, PCd4, PCu1, PCu3, PS1, PS2 and incubated at 37°C for 48 hours. Growth of the organism measured using spectrophotometer at 600 nm.

pH Tolerance

MRS Broth with pH 2, 3, 4, 5, 6 was inoculated with a 0.1ml activated culture of each bacterial suspension viz. PCd1, PCd3, PCd4, PCu1, PCu3, PS1, PS2 and incubated at 37° C for 48 hours. Growths of the organism were measured using spectrophotometer at 600 nm.

Determination of Antibacterial Activity of Isolates:

Antibacterial activities of isolates were carried out in N. agar plates by well –diffusion assay. Probiotic cultures were activated in N broth and incubated at 37° C for 24 hours for activation of cultures and then centrifuged at 3000 rpm for 15min and supernatant was collected to study antibacterial activity. Using *in-vitro* agar well diffusion method, antimicrobial activity experiments were carried out [9]. The activity of *Lactobacillus* strains against test microorganisms (0.2ml of activated test cultures 2 Gram negative and 2 Gram positive; viz. *E. coli* MTCC-425, *Salmonella typhi* MTCC-733, *Staphylococcus aureus* MTCC-96 and *Bacillus cereus* MTCC-430) was inoculated in molten agar and poured in sterile plates than allowed to solidify. Wells were prepared at equal distance in solidified agar plates using cup-borer. Overnight grown cultures of *L. acidophilus* strains were inoculated in the wells of nutrient agar whereas test microorganisms were inoculated by pour plate technique. The plates were incubated at 37°C for 24 hours. Zone of diameter was measured and then with the help of that zone index was calculated at the end of incubation period. The values of zone diameter given in Table 3, is a mean values; Standard drug used is Streptomycin.

Determination of activity index

The activity index of the probiotic culture was calculated as:

$$A.I. = \frac{\text{Mean of zones of inhibition}}{\text{Zones of standard antibiotic drug}}$$

Standard drug used was Streptomycin.

RESULTS AND DISCUSSION

On the basis of cell morphology, colony characterization and all biochemical tests performed 12 isolates (curd – 4, Cucumber – 6, Soya milk - 2) were selected for further study. These different isolates were obtained on MRS agar plates for their morphological and biochemical characterization. Isolates were selected for further isolation and purification. Ability to ferment different carbohydrates was determined on MRS-BCP broth medium, by using the different sugars as carbon source, which was added to the sterile basal medium to get final concentration 1% w/v. Carbohydrates utilization was evaluated after 24 and 48 hours. Fermentation of sugar provided to isolates studied which indicates, isolates can utilize provided sugars. Gas production from glucose was assessed by inoculation of cultures into 5 mL MRS broth containing inverted Durham tubes and incubating at 35°C for 2 days. After biochemical characterization isolates

were studied for their survival and growth in presence of different concentration of bile and acid as well as with different NaCl concentration. The isolates were subjected to the test for biochemical reactions. Colony characteristics of all the isolates revealed few common characteristics as mentioned in Table 1.

Cultural Characteristics of Isolates:

Out of 12 Isolates, 5 isolates were cocci and 7 isolates were rod in shape. Only rod shaped organisms were used to carry out further probiotic activities and characterized. Different biochemical test were performed for the primary differentiation among the isolates (Table 1).

Biochemical Characterization of Selected Isolates:

From the biochemical test performed as shown in Table 2, all test cultures were showing negative results for Starch Utilization, Citrate Utilization, Casein Hydrolysis, Gelatin Hydrolysis, VP Test, H_2S Test, Indole Production, Urea Utilization, Nitrate reduction and Catalase Test. Except all cultures, PCu1 and PS2 shows positive results for ammonia production. All cultures showed curdling property when were inoculated in sterilized milk under standard conditions. Proteiolytic, Lipolytic and Haemolytic tests are negative for each selected seven isolates.

Sugar fermentation test

Except for Rhamnose Sugar, rest sugars viz. Fructose, Galactose, Lactose, Maltose, Sucrose, Arabinose, Manose and Cellobiose are fermented by all selected seven cultures. However PCd4, PCu3, PS1 and PS2 do not ferment Glucose sugar and hence shows negative results as shown in Table 2.

Study of growth profile:

Gram positive, rod shaped and catalase negative isolates were preserved on MRS agar plates were selected for further study. Tolerances to bile and acid and growth in presence of NaCl are the most important properties for selection of potential probiotic strains.

A. Bile salt tolerance of isolates:

Bile Salt Tolerance Parameter for Growth of Isolates screened from Curd Sample

Figure 1 shows survival and growth of curd isolates in presence of different concentrations of Bile salt. All the isolates have been found to survive in presence of 1%, 2%, 3% and 4% bile salt. Isolate PCd3 shows better growth as compared to other three isolates. This indicates that PCd3 can be selected as potential candidate for the construction of a consortium for oral administration of probiotics.

Bile Salt Tolerance Parameter for Growth of Isolates screened from Cucumber Sample

Figure 2 shows survival and growth of Cucumber isolates in presence of different bile salt concentrations. Graph indicates isolate PCu3 to be better surviving consistently in presence of different bile salt concentrations as compared to PCu1.

Bile Salt Tolerance Parameter for Growth of Isolates screened from Soy Sample

Figure 3 shows Survival and Growth of Soy sample isolates at different Bile salt concentrations and shows best survival of PS1 in different bile salt concentrations. Graph shows increasing survival in 1%, 2% and 3% Bile salt concentrations while in 4% bile salt concentrations comparative less growth. No growth is observed in 4% Bile salt concentrations and in 1%, 2% and 3% Bile salt concentrations only survival is found for PS2 isolate which can be observed from graph. So it can be said that Bile may act as a stimulatory molecule as majority of the isolates show only survival at lower concentration but may restrict growth at higher concentrations. Like bile tolerance, acid tolerance is also significant character for probiotics. Intestinal tract or gut is an area where acidic pH is there. The effect of pH on the growth and bile tolerance of the selected isolates were also studied. The strains resistant to low acidic pH were screened for their ability to tolerate the bile salt. Although the bile concentrations of the human gastro intestinal tract varies, the mean intestinal bile concentrations is believed to be 2% w/v and the staying time is suggested to be 4 hours [10]. Previous studies on bile tolerance levels of *L. fermentum* were also various. The highest tolerance level of *L. fermentum* ACA-DC 179 to bile salts was 2% [11]. *L. fermentum* AD1 strain was able to grow in the presence of 1% bile and remained 75.4% viable cells after 24 h incubation [12]. *L. fermentum* SGM3, which was isolated from chicken, has a high tolerance to 0.3% bile salts as 100%. However, there is no tolerance to bile salt founded for PG3 and PGM3, which were isolated from poultry [13]. Compared to the above probiotics, *L. fermentum* F6 showed a strong bile tolerance. Since bile salts disorganize the structure of the cell membrane, it is toxic for living cells [14]. Therefore, bile tolerance is considered as an important characteristic of the *Lactobacillus* which enables it to survive, grow, and exert its

action in gastrointestinal transit. *Lactobacillus* strains which could grow and metabolize in normal physical bile concentration could survive in gastrointestinal transit [15]. Furthermore, the effect of bile salts on the survivability of different *Lactobacillus* strains depends on the concentration and the specific properties of the strains. It is well known that bile-salt concentration in the gut is not static, ranging from 1.5% to 2% (w/v) in the first hour of digestion, and decrease afterwards to around 0.3% (w/v) [16].

B. **pH tolerance of isolates:**

Another important criterion to be a good source of probiotics is the tolerance of high acid levels, which is present in our stomachs. The lowest pH recorded has been pH 1.5 [17]. Good probiotic sources should withstand at least pH 3.0 [18].

pH Tolerance Parameter for Growth of Isolates screened from Curd Sample

Figure 4 shows that each isolate of curd sample is able to grow at pH2, to pH7. Favorable growth is observed at pH5, pH6 and pH7, while at pH2, pH3 and pH4 they can survive. Survival and growth of curd isolates at low pH is significant because according to their characteristics of probiotic, can be applied for dairy industry as well as used in pharmaceutical aid.

pH Tolerance Parameter for Growth of Isolates screened from Cucumber Sample

Figure 5 shows Cucumber isolates survival and growth at different pH. It can be observed that both isolate can survive in pH 2 and pH3 while gives good growth at pH 4, pH5, pH6 and pH7. Sample PCu1 fails to survive and grow at pH2 and pH3 while PCu3 isolate survives and gives growth that can be considered as significant character of isolate to act as probiotics. Because in intestinal tract acidic pH is there and isolates should survive from the entry and to establish in gut.

pH Tolerance Parameter for Growth of Isolates screened from Soy Sample

Figure 6 shows Survival and Growth of Soy sample PS1. PS2 isolate at lower pH shows little growth. Graph shows good survival of PS1 isolate at pH2, pH3 and pH4 as well as less restricted growth of at different pH values. These findings can be justified by the fact that PS1 isolate must survive in low pH and can show its role to protect intestine from the infection by maintaining and growing at low pH.

C. **NaCl tolerance of isolates:**

Study of growth pattern and characteristics of probiotics in presence of varying concentrations of NaCl was also carried out. This was performed to check the ability of the isolates to tolerate different NaCl concentrations during their residence in the intestine.

NaCl Concentration for Growth of Isolates screened from Curd Sample

Figure 7 indicates that curd isolate not only survive but shows good growth in 1%, 2%, 3% and 4% (w/v) NaCl concentrations. As these isolates find their applications in dairy industry, their survival in presence of high salt concentration is very significant.

NaCl Concentration for Growth of Isolates screened from Cucumber Sample

Figure 8 shows Survival and Growth of Soy sample isolates at different NaCl concentrations. All Isolates gives good and favorable growth at different NaCl concentrations. Isolates shows relatively less growth at 3-4 % NaCl concentrations i.e. at higher Salt concentrations. This variation in the pattern of growth and survival in the presence of different salt concentratins can be attributed to them being the different strains.

NaCl Concentration for Growth of Isolates screened from Soy Sample

Figure 9 shows Survival and Good Growth of Soy isolates at different NaCl concentrations. Isolates shows good and favorable growth at different NaCl concentrations. Survival and Growth in presence of NaCl shows that these organisms can combate the pathogens and can stop their establishment in presence of high salt concentrations.

Antibacterial Activity of Isolates:

An important aspect of the function of probiotic bacteria is the protection of the host gastrointestinal microenvironment from invading pathogens. It is generally believed that the resident gastrointestinal microflora in vivo provides protection for the host against possible Colonization by pathogenic bacteria [19]. Antibacterial activity of isolates was carried out on N-agar plates by well –diffusion assay against *E. coli* MTCC-425, *Salmonella typhi* MTCC-733, *Staphylococcus aureus* MTCC-96 and *Bacillus cereus* MTCC-430 species. Cultures were activated in N broth. Isolates inhibits the above mentioned organisms or not was studied and zone of inhibition was measured in terms of

zone diameter and with the help of that zone index was calculated where streptomycin was used as standard. All cultures were procured from Culture center Pune.

Table 3, shows that PCd1, PCd3, PCd4, PS1, PS2 isolates have antimicrobial activity against *E.coli* and *Salmonella*, and were not showing any zone for *S. aureus* and *B. cereus* species. *S.aureus* is more inhibited by PS1 as compared to PS2, while contrastingly PS1 and PS2 are unable to inhibit *B. cereus*. Production of antimicrobial substances is another mechanism by which probiotics may protect against infections. Production of acidic metabolites such as lactic and acetic acid and the pH reductive effect is one example. [20].

Each isolates shows comparative inhibition pattern and it can be observed that PS1 gives antimicrobial activity against almost test culture except *B. cereus*. *Lactobacillus* spp. has demonstrated inhibition of growth of human pathogens due to acid production. [20]. Naturally fermented milk products are usually considered safe because of the low pH and production of antimicrobial substances by fermenting organisms [21]. The effect of Lactobacillus in controlling the proliferation of pathogenic bacteria in the traditional naturally-fermented dairy products has been well reported. Lactobacilli produce a wide range of antibacterial compounds including sugar catabolites such as organic acids (e.g., lactic acid and acetic acid); oxygen catabolites such as hydrogenperoxide; proteinaceous compounds such as bacteriocins, other low-molecular-mass peptides, and antifungal peptides/proteins; fat and amino acid metabolites such as fatty acids, phenyllactic acid, and OH-phenyllactic acid; and other compounds such as reuterin and reutericyclin [22].

As one of important mechanisms to exert probiotic properties, a great deal of researches focused on the antimicrobial substances production [20]. This study was conducted to determine the presence of antimicrobial activities among the probiotics incorporated into these different food products against common microbial pathogens. Substantiating the antimicrobial activities of probiotics will affirm their use in the development of functional foods for the betterment of the health of the consuming public. The economic success and exciting prospects of probiotic products have accelerated research on intestinal flora. Thus from the study of antibacterial activity of isolates obtained from different samples that their applications in the field of biology may show new horizons and isolates could be useful as a supplementary which work in harmony with the different tissues and organ cells to metabolize proteins and aid to get rid of poisonous excesses from our body.

CONCLUSIONS

Natural food samples are known to be good sources of microorganisms of our interests. The study reveals such facts and serves some of the natural food sample viz. curd, soy and cucumber as the natural sources of bacteria having probiotic properties. These microorganisms serve to have the probiotic properties and exhibit tolerance to wide pH Values, bile concentrations and NaCl Concentration as well. However most of them also show the antibacterial activities indicating their medicinal values. None of the assayed strains showed hemolytic, proteolytic or lipolytic activity indicating their status as safe cultures for formulating probiotic foods.

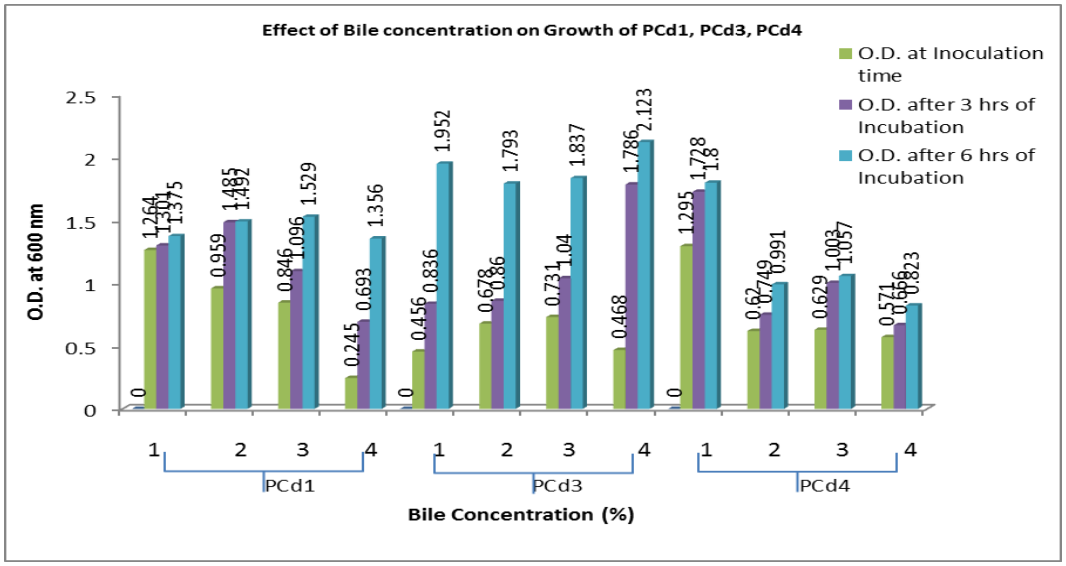

Fig. 1: Effect of bile concentration on Growth of PCd1, PCd3, PCd4

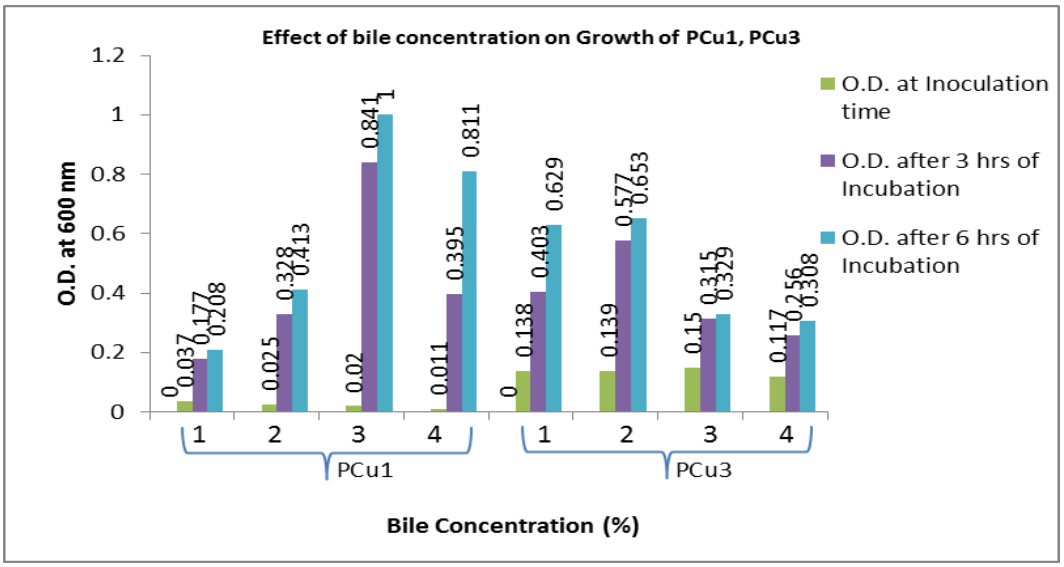

Fig. 2: Effect of Bile Salt Concentration on Growth of PCu1, PCu3

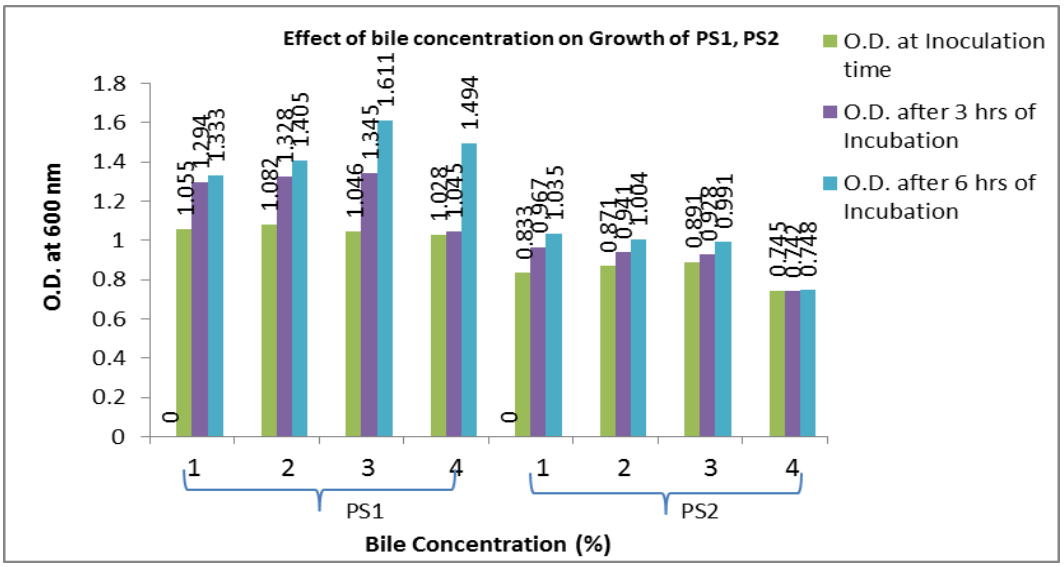

Fig. 3: Effect of bile concentration on Growth of PS1, PS2.

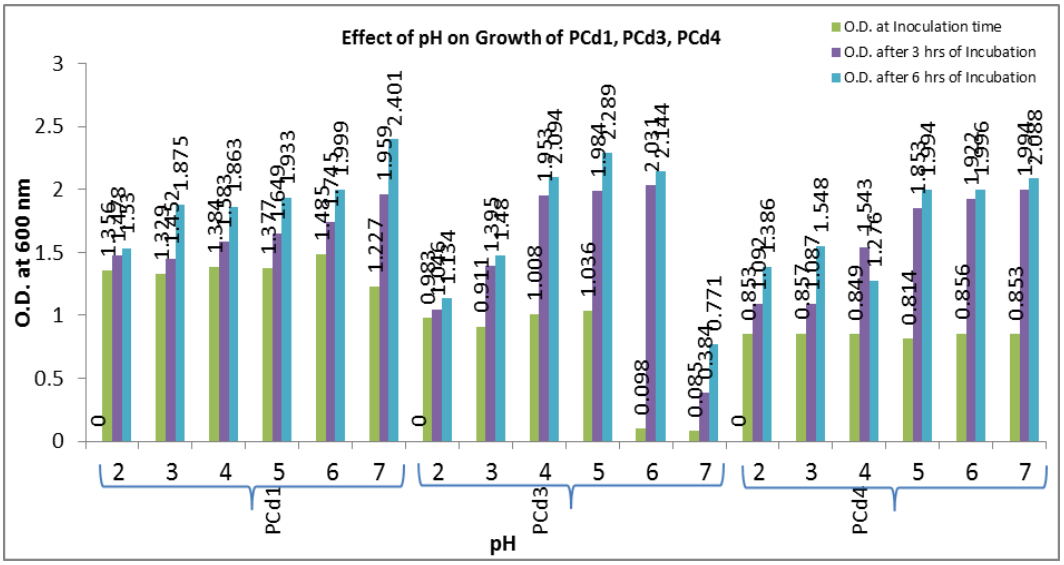

Fig. 4: Effect of pH on Growth of PCd1, PCd3, PCd4

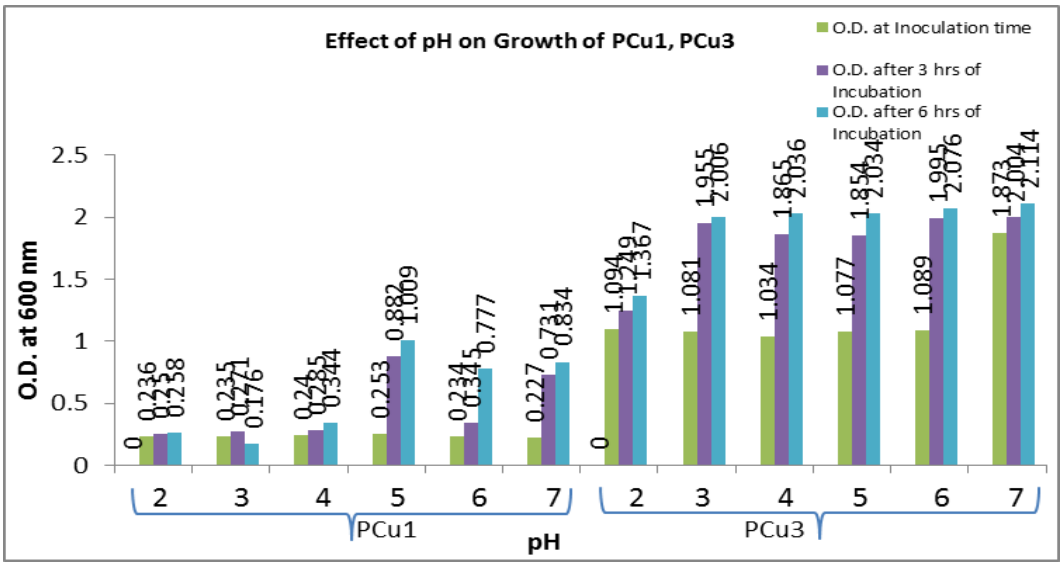

Fig. 5: Effect of pH on Growth of PCu1, PCu3

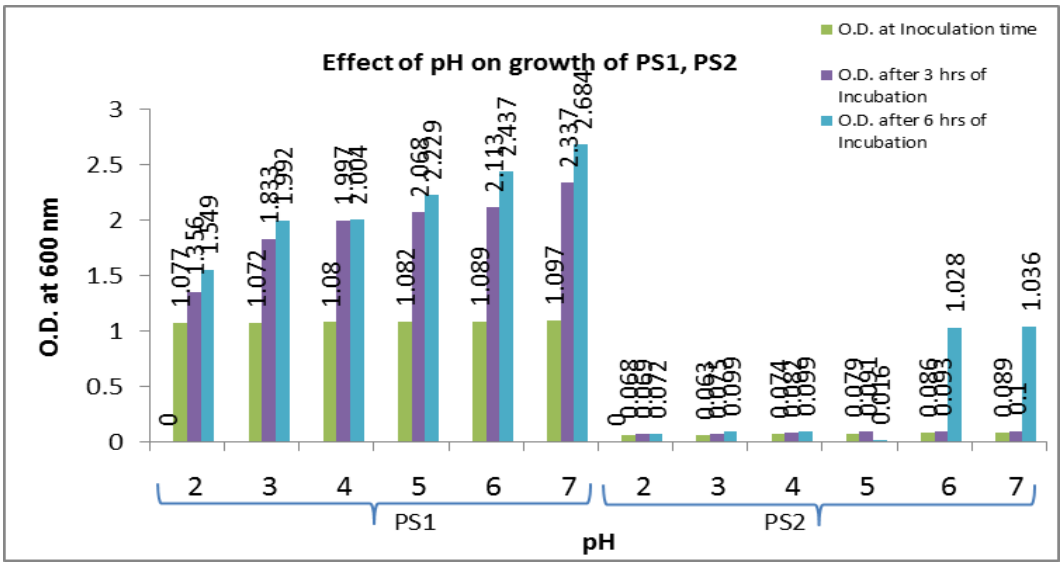

Fig. 6: Effect of pH on Growth of PS1, PS2

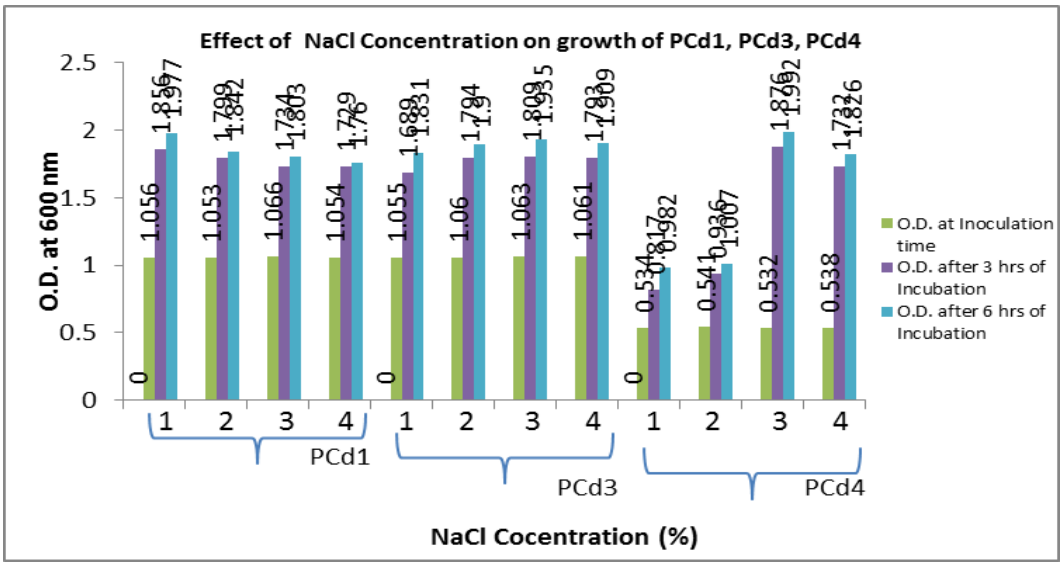

Fig. 7: Effect of NaCl Concentration on Growth of PCd1, PCd3, PCd4

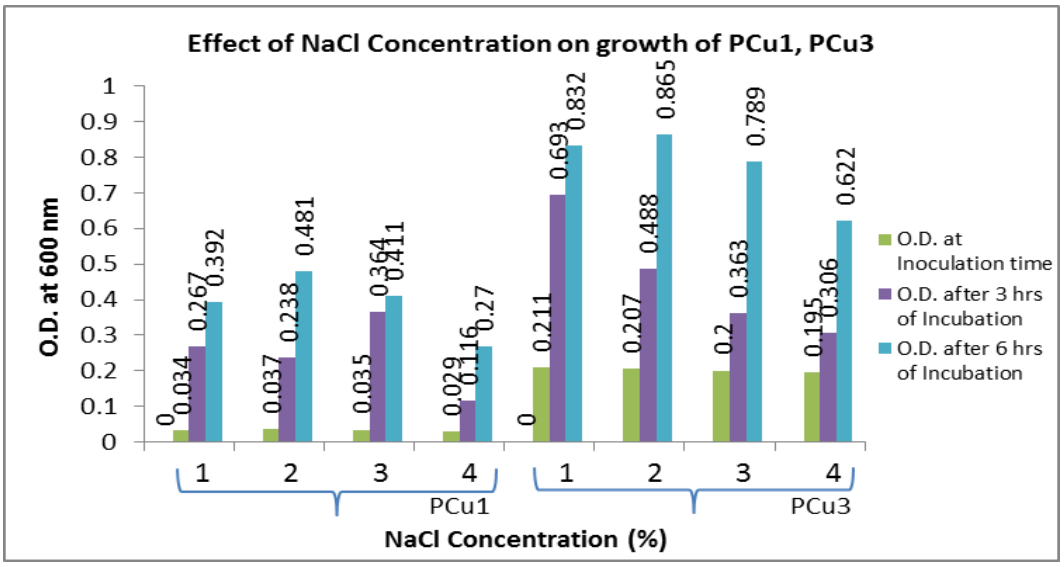

Fig. 8: Effect of NaCl Concentration on Growth of PCu1, PCu3

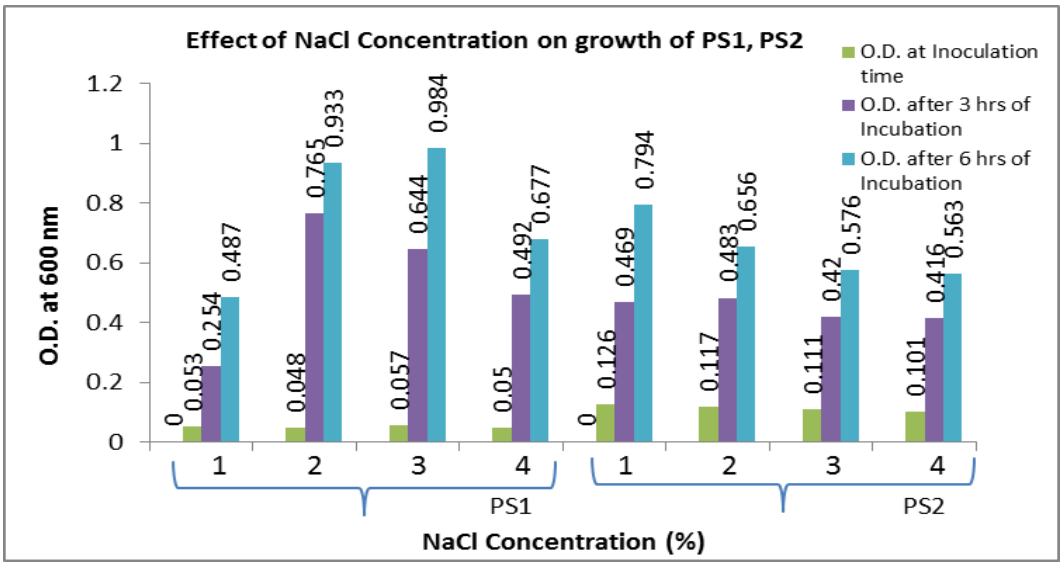

Fig. 9: Effect of NaCl Concentration on Growth of PS1, PS2

Table 1: Cultural Characteristics of Isolates from Natural Food Sample:

Cultural characteristics												
Source	Curd				Cucumber						Soy milk	
Character	PCd1	PCd2	PCd3	PCd4	PCu1	PCu2	PCu3	PCu4	PCu5	PCu6	PS1	PS2
Size	Small	Medium	Medium	Medium	Small	Medium	Large	Medium	Medium	Medium	Small	Medium
Shape	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Smooth	Smooth
Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Rough	Rough	Rough	Smooth	Regular	Regular
Elevation	Flat	Flat	Flat	Flat	Raised	Raised	Flat	Flat	Flat	Flat	Flat	Flat
Surface	Wet	Moist	Viscous	Wet	Moist	Moist	Dry	Moist	Moist	Moist	Dry	Dry
Consistency	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Powdery	Viscous	Viscous	Viscous	Powdery	Powdery
Opacity	Opaque	Clear	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Pigment	White	White	White	White	White	Off-white	White	Off-white	White	White	White	White
Microscopic characteristics												
Staining	PCd1	PCd2	PCd3	PCd4	PCu1	PCu2	PCu3	PCu4	PCu5	PCu6	PS1	PS2
Gram's	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Endospore	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Capsule	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Shape	Rod	Cocci	Rod	Rod	Rod	Cocci	Rod	Cocci	Cocci	Cocci	Rod	Rod

Table 2: Biochemical Characteristics and Sugar Fermentative Test of Isolates:

Sr. No.	Biochemical Test	Organism Code						
		PCd1	PCd3	PCd4	PCu1	PCu3	PS1	PS2
1	Starch Utilization	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
2	Citrate Utilization	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
3	Casein Hydrolysis	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
4	Gelatin Hydrolysis	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
5	MR Test	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
6	VP Test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
7	H_2S Production	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
8	Indole Production	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
9	Ammonia Production	-Ve	-Ve	-Ve	+Ve	-Ve	-Ve	+Ve
10	Nitrate Reduction	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
11	Urea utilization	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
12	Catalase Test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
13	Curdling Test	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
14	Proteiolytic Test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
15	Lipolytic Test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
16	Haemolytic Test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Sugar Fermentation Test								
1	Glucose	+	+	-	+	-	-	-
2	Fructose	+	+	+	+	+	+	+

3	Galactose	+	+	+	+	+	+	+
4	Lactose	+	+	+	+	+	+	+
5	Maltose	+	+	+	+	+	+	+
6	Sucrose	+	+	+	+	+	+	+
7	Arabinose	+	+	+	+	+	+	+
8	Manose	+	+	+	+	+	+	+
9	Cellobiose	+	+	+	+	+	+	+
10	Rhamnose	-	-	-	-	-	-	-

Table 3: Antibacterial Activity of Isolates:

	E. coli (MTCC - 425)		*Salmonella typhi* (MTCC - 733)		*S. aureus* (MTCC - 96)		*Bacillus cereus* (MTCC - 430)	
Culture Code	Zone of Inhibition (mm)*	Activity Index (A.I.)	Zone of Inhibition (mm)*	Activity Index (A.I.)	Zone of Inhibition (mm)*	Activity Index (A.I.)	Zone of Inhibition (mm)*	Activity Index (A.I.)
PCd1	14	0.5833	12	0.5000	---	0.0000	---	0.0000
PCd3	14	0.5833	14	0.5833	---	0.0000	---	0.0000
PCd4	16	0.6666	17	0.7083	---	0.0000	---	0.0000
PCu1	---	0.0000	---	0.0000	---	0.0000	---	0.0000
PCu3	---	0.0000	---	0.0000	---	0.0000	---	0.0000
PS1	23	0.9583	15	0.6250	34	1.4166	---	0.0000
PS2	12	0.5000	11	0.4583	12	0.5000	---	0.0000

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