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RESEARCH ARTICLE

PRODUCTION OF FOLATE BY LACTOBACILLUS AND BACILLUS CEREUS ISOLATED FROM COW MILK

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ABSTRACT

Lactic acid bacteria (LAB) is defined as live microorganism, when administered in adequate amount confer a health benefit on the host. Raw cow milk was serially diluted, spread plated, sub cultured and pure cultured. Screening of isolates (MK-2 and MK-4) was performed on the basis of zone formation and characterized. The morphological and biochemicalcharacterization was carried out and it was identified as MK-2 *(Lactobacillus sp*) and MK-4 *(Bacillus cereus*). Both the organism were screened for Folateproduction by inoculating the microorganism in folate production media. The highest Folatedetected for MK-2 *(Lactobacillus)*, followed by MK-4 *(Bacillus cereus*) revealed 150 µg/ml of Folate. In strain MK-4 showed 50-60 µg/ml of Folate production. Further study extended for Folateproducing lipase enzyme from MK-2 and MK-4 revealed negative resultfor lipase. Whereas, positive result was obtained for protease enzyme by MK-2 and MK-4. Antimicrobial activity of *Lactobacillus*against selective bacterial pathogens revealed maximum activity was observed against *Pseudomonas aeruginosa, E.coli, Staphylococcus aureus,* and *Shigella flexneri*. The antifungal activityrevealed maximum activity against *Aspergillus niger* and *Aspergillus flavus*. Probiotic properties of *Lactobacillussp*tested against different concentration of ox bile with MK-2 and MK-4 strain showed resistance against ox bile. The strain MK-4 showed maximum survivability. In another study to assess the probiotic property such as NaCl tolerance, MK-4 strain showed maximum tolerance of6.5%of NaCl. MK-2 showed maximum growth at pH7. The total protein content of folic acid (bacteriocin) produced by MK-2 (*Lactobacillus sp*) revealed 2.01µg/ml, and the total protein content ofMK-4 showed 1.97 µg/ml protein content respectively. Determination of molecular weight of folic acid for the strain (MK-2 and MK-4) revealed 46 KDa and 30 KDaby SDS PAGE analysis. HPLC analysis was performed to analyze the metabolite present and it was confirmed with prominent peaks with retention time of 1.801 indicate the folic acid metabolite.

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INTRODUCTION

Probiotics are live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. The term "Probiotics" was originally used by Lilly and Stillwell, (1965) to mean a substance that stimulates the growth of other microorganisms. Folateisanessential component of the human diet. It is involved, as a cofactor, in many metabolic reactions, including biosynthesis of the building blocks of and DNA and RNA. (Vander Put, *et al*., 2001). In order to elucidate the basis for the assessment of the health promoting potential of probiotics. Vitamin B9 (folic acid and folate inclusive) is essential to numerous body function.

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The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reaction involving folate (Wollf, *et al*., (2009).A large number of epidemiological studies have provided evidence that adequate intake of folate may prevent the neural tube defects, coronary heart disease and several types of cancer. Afolate level set at 200mcg per day is required for human body but it was advocate by several experts in increase the consumption of folate especially for pregnant women are known to double intake of folate, recommendations in most country are therefore set to 400 mcg per day. Hence in the present investigation, to collect the raw cow milk, and to isolate the microorganisms by serial dilution and to spread plate, subculture and pure cultureexperiments was performed. Screening the efficient folate producing microorganism *Lactobacillus sp*and *Bacillus cereus*was carried outby assessing zone formation and the isolate were characterized by

morphological and Biochemical test. Antimicrobial and antifungal activity of Folic acid producing microorganisms (MK-2 and MK-4) by studied well diffusion method with prepared with production media supplemented with casein, Riboflavin, Niacin, Dextrose, and other salts. The enzyme activity for folic acid producing microorganisms and to evaluate the probiotic properties such as NaCl tolerance, Ox bile salts, acidic pH for folic acid producing microorganism was performed. Besides, the protein content of folic acid producing microorganisms in production media was estimated and determination of the protein profile by SDS-PAGE was performed. Determination of folic acid by HPLC analysis was carried out.

MATERIAL AND METHODS

Collection of Sample

The sample was collected from raw cow milk. Commercial products like milk and curd were also collected and screened for folic acid production.

Isolation of *lactobacillussp* Frommilk and Curd

About 1ml of the milk and curd sample was serially diluted in 99ml distilled water. A suitable dilution of this sample was spread plated on MRS agar plates. The plates were incubated at 37^0c and the number of the colonies was counted after 24-48 hours of incubation. The morphologically distinct colonies were selected and streaked on MRS agar slants for further study.

Screening of folate producing microorganisms

Screening of folate producing microorganisms were performed using the modified basal medium with folate (100mg) as the source. The isolate were streaked on the plates and the plates were incubated at 37^0c for 48 hours. The strains that produced clearing zones in the medium was selected as folate producing microorganisms.

Identification and Characterization of the efficient folate producing microorganism by morphological and biochemical characterization

Morphological characteristics such as abundance of growth, pigmentation, optical characteristic, size and shape were studied on nutrient agar plates.The folate enzyme producing efficient microorganism were characterized by following test such Indole test, MR-VP test, Citrate utilization test, Triple sugar iron test, Starch hydrolysis test, Gelatin hydrolysis test, nitrate reduction test, Carbohydrate fermentation test were carried out.

Production of folic acid

The production media was Dextrose- 9 mg, Sodium citrate- 12 mg, L-Cystein- 6.05 mg, Tryptophan- 10.21 mg, Thiamine hydrochloride- 15.04 mg, Riboflavin -18.81mg, Niacin - 6.15,Dipotassium phosphate-8.71 mg, magnesium sulfate-6.01mg, Sodium chloride-2.92 mg, ferrous sulfate-7.59mg, manganese sulphate -7.55mg, Mono potassium phosphate-6.80mg, this chemicals were add in 100ml distilled water and media was used for further experiments.

Screening for protease and lipase enzyme activity for folic acid

All the isolates were screened for their ability for hydrolysis of protein. The cultures were inoculated in to MRS agar which is supplemented with 10% skim milk agar and the inoculated cultures were incubated at 37^0c for 24- 48 hours. Colonies were observed for clear zone. All the isolates were screened for the ability to produce lipase enzyme. The cultures were inoculated in to MRS agar which is supplemented with 1% of tributyrin glycerol and the inoculated cultures were incubated at 37^0 c for 48hours. Colonies were observed for opaque zone.

Antimicrobial and antifungal activity of compounds treated against bacterialand fungal pathogens

Antimicrobial activity of the extract of compounds was determined using well diffusion method. It was performed by sterilizing in Mueller Hinton agar media. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test bacterial pathogens were swabbed onto the surface of Mueller Hinton agar plates. Wells were impregnated with 25 µl of the test samples. The plates were incubated for 30 min to allow the extract to diffuse into the medium. The plates were incubated at 30^oC for 24 hours, and then the diameters of the zone of inhibition were measured in millimeters. Each antibacterial antifungal assay was performed in triplicate and mean values were reported. Each antifungal assay was performed in triplicate and mean values were reported.

Probiotic Properties of Isolates

The determination of probiotic properties of isolates were performed by the following methods.

Selection of acid Producing Bacteria

The isolation of acid producing bacteria, was carried out by MRS medium supplemented with $CaCo_3$ and L-cystein. The isolated organisms were tested for acid production by streaking on the plate. Incubate the plates at 37^0C for 24-48 hours. A clear zone around the isolates indicate the positive result.

Tolerance to sodium chloride, acidic phand bile salt

MRS broth was prepared with different concentration of NaCl i.e., 4, 5, 6, 6.5% and it was then inoculated with the isolated organism and incubated at 37^0C for 48 hours. The turbidity was measured at 600 nm for the ability to tolerate NaCl.Determinations of resistance to P^H were used invitro. The tolerance to acidic pH was tested by inoculating 1% (v/v) fresh over night culture of MK-2 and MK-4 in to the MRS broth with varying pH ranging from 2.5-9.0. The pH were adjusted with concentrated acetic acid (99%) and 5 N NaOH. The inoculated broths were incubated at 37^oC for 24 hours. After 24 h of incubation growth of the bacteria were measured using a spectrophotometer, reading the optical density at 560 nm (OD) against the uninoculated broth. Bile salt resistance was performed by using ox bile at different concentration (0.0, 0.3, 0.5, and 1.0%).

Partial Purification of Bacteriocin

The isolated strain was grown in MRS broth (pH 6) seeded with 5% inoculum of overnight culture and maintained at 30^oC

for 48 hours. Partial purification of bacteriocin was done by salt saturation method.

Quantitative Estimation of Protein

The quantitative estimation of protein was determined by the method of Lowry *et al*., (1951), using bovine serum albumin as the standard.

Protein estimation of whole cell extract

About 1ml sample of whole cell extract was taken and 0.2 ml of alkaline copper reagent was added. It was incubated at room temperature for 10 minutes. To that, 500µl of diluted Folin's reagent (1:1 with distilled water) was added. The test tubes were incubated at room temperature for 30 minutes. Sample was read at the absorbance of 660 nm at optical density.

$$\text{Protein (mg/ml)} = \frac{\text{O.D of the sample X Concentration of Standard X O.D of the standard}}{\text{ml of Sample used}}$$

Determination of molecular weight of protein in sds-page

The molecular weight of the crude enzyme was determined by sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-Page). Polyacrylamide gel electrophoresis was done to separate protein. The crude enzyme was separated using SDS-PAGE.

Folate analysis in milk by hplc

The sample for MK-2 was analyzedforfolate production by HPLC method. To prepare cell free extract MK-2 sample was centrifuged at 10,000 rpm for 10minutes and the supernatant was collected and mixed with methanol and injected into HPLC system.

RESULTS

Isolation and characterization of microorganisms from Milk Sample

The samples were collected fromraw cowmilk Madurai. The samples were serially diluted on MRS agar medium (Plate-1). This specific medium was used for the isolation of lactic acid bacteria for folic acid producing microorganisms. The efficient strains were subjected to screen for their ability to produce folic acid by growing them in the medium contain folic acid and casein agar plates were incubated at 30^0C for 24 hours. Among the 5 strains the efficient strains was found to be MK-2 and MK-4. The isolated strains (MK-2 and MK-4) was identified based on its physiological and biochemical characteristics features. On MRS agar medium, the isolate showed abundant growth and it produced large, white, irregular colonies. The results were noted in Plate: 2, 3 and 4.

Identification of folate producing microorganisms

Evaluation of health related beneficial functions of two strains MK-2 and MK-4 from milk fermented isolates in commercial dairy products and foods is very important task in present study. The two different strains were inoculated into MRS agar medium. Lateridentified by Morphology and Biochemical characterization. Owing to better growth in folic acid medium which is an indicates of folate production. The biochemical characterization of MK-2 and MK-4 was initiated by gram staining. The MK-2 isolate showed abundant growth and it produce large white irregular colonies in MRS medium. The isolate MK-2 was found to be gram positive, Non motile organism and in does not produce spore. From the results it was confirmed that the isolatestrain belong to *Bacillus sp*. The isolate MK-4 was found to be gram positive with short rods, occurring single colony on MRS agar which were found to be large, raise opaque, irregular margins. Indole was not produced by the isolate MK-4.From the results, it was observed the organism belonged to genus *Bacillus sp*. The results were compared in accordance with Bergey's manual of determinative bacteriology. Further this isolate was confirmed as *Bacillus* at genus level, followed by species level as *cereus*. The further confirmation was performed by various test. Depicted in the Table:1 and Fig:1.

Production of folic acid producing microorganisms

The fermented milk is a good source for the growth of Lactic acid bacteria and *Bacillus cereus*. These strains which has the ability to produce folic acid by growing them in the chemically defined medium and incubated at 30^0C under shaking condition (210 rpm).The incubation time is one of the significant factors influencing the folate levels and in this study samples were exposed to 24hours to 30 hours. A total folate included a sum of enzymatic deconjucation intra and extracellular folate. Therefore the highest total folate level was detected on MK-2 *(Lactobacillus sp)* and (MK-4) (*Bacillus cereus*) did not produceadequatefolate and it also been reported that the only production of folate was detected in MK-2 strains which was highly responsible for folic acid production. The MK-2 showed highest folate production of 150µg/ml in a folate medium. Similarly MK-4 strain was showed relatively less production of 50-60 µg/ ml respectively. (Plate:5).

Screening of protease and lipase enzyme activity for folic acid production

The efficient folate producing microorganisms were screened for their ability to produce an enzyme called protease. Among the five isolates (MK-1, MK-2, MK-3, MK- 4, and MK-5) only one microorganisms (MK-2) led produced protease, which is confirmed by the clear zone formation exhibiting 17mm.The results were exhibited in (Table:2 and Plate:6). The source may be the reason for utilized the protease production of the isolates from folate producing microorganisms. The study was further extended to checkout, thefolate producing lipase enzyme. The isolated colonies were MK-2 and MK-4 inoculated in to MRS agar medium. Both strains revealed negative result for lipase production on depicted in Plate:7.

Determination of antimicrobial and antifungal activity of folic acid producing microorganisms

The susceptibility of various clinical pathogens to growth inhibition by the folic acid producing microorganisms (MK-2 and MK-4) was presented in Plate: 8 and Figure:2. The antimicrobial activity of the organism was detected by adding 50µlof supernatant into the well. After incubation, zone formation was measured. It showed inhibitory activity against *E. coli,Staphylococcusaureus, Pseudomonas aeruginosa* and *Shigella flexneri*. Among these, maximum activity was observed against *Pseudomonas aeruginosa, E. coli* and

Staphylococcus aureus and minimum activity was observed against *staphylococcus flexneri.* The susceptibility of various fungal pathogens to growth inhibition by the Folic acid produced microorganisms was presented in Table and Plate. The antifungal activity of the fungal pathogen was detected by adding supernatant in to the well 72 hours zone formation was measured. It showed inhibitory activity against *Aspergillus nigerand Aspergillus flavus*. Among these fungal pathogen maximum activity was observed against *Aspergillusniger*and minimum activity was observed against *Aspergillusflavus* (Plate: 9 and Fig: 3).

Selection of strain for acid Production

The isolates MK-2 and MK-4 were grown on MRS medium supplemented with 0.05% L-cystein and 0.5% calcium carbonate for its acid production. A clear zone was produced around the colony indicated the production of acid.

chloride. Simultaneously, isolate MK-4 showed maximum tolerance at 4% and it was found to revealed an optimum growth up to 6.5% of sodium chloride. The ability of the strains (MK-2 and MK-4) able to survive in bile salt is a very important in probiotic studies. The test organismexhibited the resistance against different concentration of ox bile. The isolated lactobacillus was able to survive in 0%, 0.3%, 0.5%, and 1% bile salt. The isolated lactobacillus species (MK-2) was also able to multiply in the above mentioned concentrations of bile salt (Fig: 5).The strain MK-4 showed maximum survivability in the bile salt which again proved their probiotic ability.

Partial Purification of Bacteriocin: Partial purification of bacteriocin was performed by precipitation of cell free supernatant with 70% ammonium sulphate. Precipitation was found to increase the level of bacteriocin. The *Lactobacillus species* (MK-2) mainly responsible for bacteriocin production.

TABLE: 1.MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE ISOLATE

S.NO	TESTS	OBSERVATION	
		MK-2	MK-4
1.	Grams staining	Positive	Positive
2.	Spore staining	Negative	Positive
3.	Indole test	Negative	Negative
4.	Methyl red test(MR)	Positive	Positive
5.	Voges proskauer test	Negative	Negative
6.	Citrate utilization test	Positive	Positive
7.	Triple sugar iron test (TSI)	Positive Alkaline slant	Positive Alkaline slant
8.	Gelatin hydrolysis	Negative	Positive
9.	Nitrate reduction test	Negative	Positive
10.	Starch hydrolysis test	Negative	Positive
CARBOHYDRATE FERMENTATION TEST			
11.	Sucrose	Positive	Negative
12.	Dextrose	Positive	Positive

TABLE: 2. SCREENING OF PROTEASE AND LIPASE ENZYME ACTIVITY BY FOLIC ACID PRODUCING MICROORGANISMS

Name of strain	Zone formation of protease activity in mm	Zone formation of lipase activity in mm
MK-2	17mm	9mm
MK-4	13mm	7mm

TABLE: 3. TOLERANCE OF SODIUM CHLORIDE OF ISOLATES

S.NO	Concentration of NaCl	MK-2	MK-4
1.	3%	++	+
2.	4%	++	++
3.	5%	+	+
4.	6%	+	+
5.	6.5%	+	+

Note:++ good growth, + visible growth

Tolerance of Acidic p^{h}, sodium chloride and bile salt

Maximum growth (MK-2 revealed 2.20 ml) of isolated lactobacillus from milk fermented sample was observed at pH 5.0. The OD value was the average value of two readings, where a control OD was 0.60. The results were depicted in Fig: 4. Similarly the MK-4 strain were maximum growth at pH-7 exhibited 2.16. The results were depicting in Table: 3. MK-2 isolates revealedvariation in the NaCl tolerance. It depends upon the species. The isolated MK-2 tolerate up to greater saline condition. From Table:3, it was found that the isolated MK-2 showed maximum tolerance at 3% and 4% and it was found to show an optimum growth up to 6.5% of sodium

It was produced highest amount of bacteriocin. Whereas decreased content of bacteriocin was noted for the strain MK-2.

Determination of protein by lowrys method: The amount of protein present in the given sample (MK-2 and MK-4) was quantitatively estimated by lowrysmethod using bovine serum albumin as standard. The amount of protein present in the given sample was (MK-2) found to be-2.01 mg protein/ml and (MK-4) found to be 1.97 mg protein/ml (Fig. 6).

Molecular Weight Determination by sds-page: The molecular weight of the protein was determined by SDS-PAGE gel electrophoresis.

PLATE: 1. ISOLATION OF MILK FERMENTATED SAMPLE BY SPREAD PLATE METHOD

PLATE:2. SUBCULTURE OF LACTIC ACID BACTERIA ON MRS AGAR MEDIUM

PLATE:3. PURE CULTURE OF FOLATE PRODUCING MICROORGANISMS

PLATE: 4. SCREENING OF FOLATE PRODUCING MICROORGANISM ON FOLIC CASEIN AGAR MEDIUM

PLATE:5. PRODUCTION OF FOLIC ACID PRODUCING MICROORGANISM (MK-2 AND MK-4)

PLATE: 6. SCREENING OF PROTEASE ENZYME FOR MK-2 AND MK-4

PLATE: 7. SCREENING OF LIPASE ENZYME FOR MK-2 AND MK-4

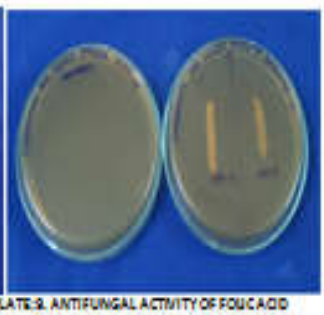
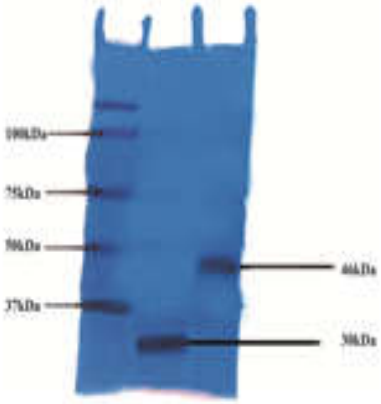


PLATE: 8. ANTIMICROBIAL ACTIVITY OF FOLIC ACID TREATED AGAINST CLINICAL PATHOGENS (MK-2 AND MK-4)

PLATE:9. ANTIFUNGAL ACTIVITY OF FOLIC ACID TREATED AGAINST FUNGAL PATHOGENS (MK-2 AND MK-4)

PLATE: 10. DETERMINATION OF MOLECULAR WEIGHT BY SDS-PAGE (MK-2 AND MK-4)



LANE: 1. PROTEIN MARKER, LANE: 2. MK-2 AND LANE: 3. MK-4.

FIG:-1. SCREENING ASSAY FOR FOLATE PRODUCING MICROORGANISM

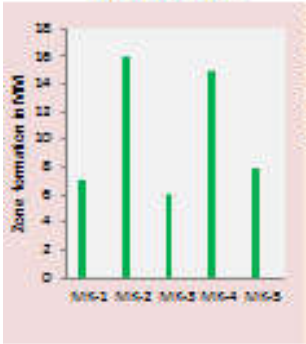


FIG:-2. ANTIMICROBIAL ACTIVITY OF THE COMPOUNDS TREATED AGAINST BACTERIAL PATHOGENS

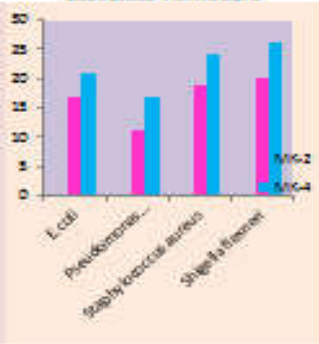


FIG:3.ANTI FUNGAL ACTIVITY OF THE COMPOUNDS TREATED AGAINST FUNGAL PATHOGENS

FIG:4: TOLERANCE TO ACIDIC pH

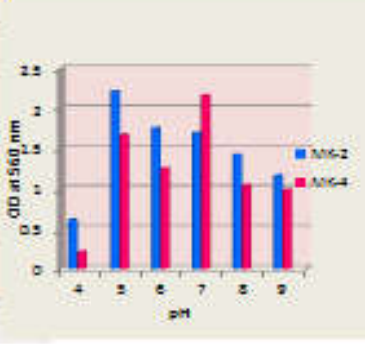


FIG:5. TOLERANCE OF OX BILE

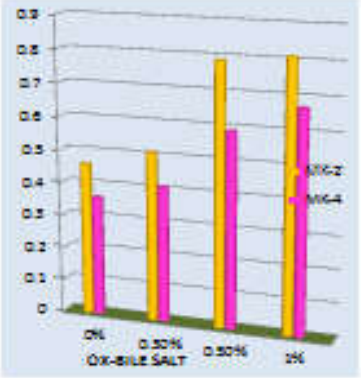


FIG: 6. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

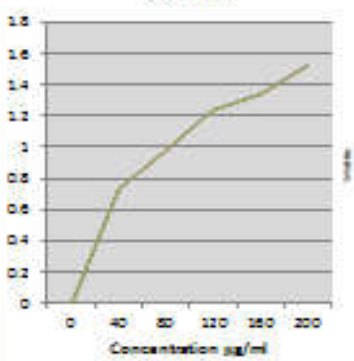
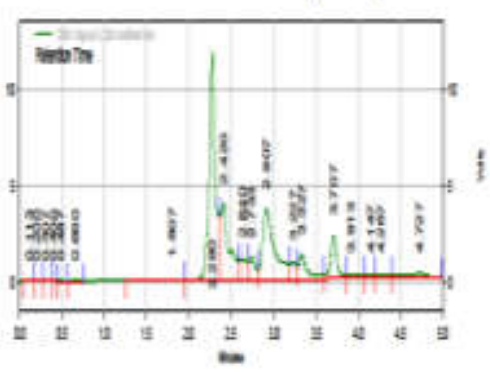


FIG:-7. HPLC ANALYSIS OF FOLIC ACID(MK-2)



The both sample (MK-2 and MK-4) protein band was observed when stained with coomassie blue and it clearly indicated the purity of the protein. The molecular weight of the partially purified protein was calculated compared with protein marker Mk-2which exhibited 46KDa and MK-4 revealed 30 KDa and protein marker 100 KDa respectively. The results were exhibited in Plate-10.

HPLC analysis of folic acid producing microorganism

The HPLC chromatogram revealed better reproducibility. A prominent peak with the retention time of 1.807 the concentration of specked standards within area of 3.363 indicate the presence of folic acid. These results demonstrate the impurities may also interfere with detection of vitamin B_9. Which appear at peak of265.Vitamin B_6(folic acid) is best detected 220 wavelength of HPLC.Similar study was carried out. The HPLC method for determination offolate in various food matrices. The presentstudy providea new data on folate concentration in raw cow milk which can be used for food data bases (Fig. 7).

DISCUSSION

Lactic acid bacteria was defined as 'live microorganisms'when administered in adequate amounts confer a health benefit on the host. There is an increased experimental evidence to suggest that probiotics can be used in the treatment and prevention of infections and chronic inflammatory disorders of the gastrointestinal tract. The application of probiotics as a functional food is of continually increased significance to human health each year. One purpose of using probiotics as a biological product is for prevention of human cancers such as colon cancer (Commane *et al.,* 2005). The fermented milk is a good source for the growth of lactic acid bacteria. *Lactobacillus* create healthy and beneficial environment and protect from potentially harmful microorganisms. The samples were collected from Raw and fermented milk. The samples were serially diluted on MRS agar medium. The folic acid producing microorganism of lactic acid bacteria. The isolated strains five isolates (MK-1, MK-2, MK-3, MK- 4, and MK-5) were subjected to screen for their ability to produce folic acid medium contain folic acid.Besides, medium containing folic acid and incubated at 30^0C for 24 hours.Out of five strainsidentified the efficient strain was found to be MK-2 and MK-4. The isolated strains (MK-2 and MK-4) was identified based on its physiological and biochemical characteristics. The results it was conformed that the isolated strain belong to *Lactobacillus sp*(MK-2). Similarly MK-4 the organism belonged to genus *Bacillus cereus*.

The Raw and fermented milk is a good source for the growth of Lactic acid bacteria *lactobacillus sp*and *Bacillus cereus*. These strains possessing ability to produce folic acid by growing them in the chemically defined medium. The incubation time is one of the significant factors influencing the folate levels and in this study samples were 24hours to 30 hours. A total folate included sum of enzymatic deconjucation intra and extracellular folate. Therefore the highest total folate level was detect in strain MK-2 (*Lactobacillussp*) and the strain MK-4 (*Bacillus cereus*) did not produce folate and it also been reported that the only production of folate was detected in MK-2 and its highly responsible for folic acid producing microorganisms. The MK-2 showed highest folateproduction of 150 µg/ml in a folate medium. Similarly MK-4 strain was showed relatively decreased production of 50-60 µg/ ml respectively. The folate contents in yoghurts commercially available in the netherlands varied between less than 2 and more than 10 µg/100g. (Zulu , Dillon etal., 1997). The efficient folate producing microorganisms were screened for their ability to produce an enzyme called protease. (MK-2) lead the ability to produce protease which is confirmed by the clear zone formation exhibiting 17mm of zone produced. Similarly, the study was further extended to assess the production of lipase enzyme. These strains revealed negative result in lipase production. Maragkoudakis *et al*., (2006) also reported that potential probiotic LAB strains isolated from dairy products could inhibit the growth of H. pylori, E. coli and S. typhimurium. Some of these pathogens was found associated with causing cancers, such as H.pyflori with gastric and colon cancer (Sybesma, *et al*, 2003). The present study onsusceptibility of various clinical pathogens to growth and inhibition by the folic acid producing microorganisms (MK-2 and MK-4) wascarried out.

The folic acid producing microorganismwere determined for it is antimicrobial activity and antifungal activity for isolate MK-2 and MK-4, respectively. Similarly antifungal activity against Folic acid inhibitory activity against *Aspergillus niger*MK-2 and MK-4 on with showed zone of inhibit (20mm and 17mm) *and Aspergillus flavus* (22 and 19mm*)*. Among these Fungal pathogen maximum activity observed against *Aspergillusniger* and minimum activity was observed against *Aspergillusflavus*. (Hammes and Voger, 1995). The isolates MK-2 and MK-4 were grown on MRS medium supplemented with 0.05% L-cystine and 0.5% calcium carbonate for its acid production. A clear zone was produced around the colony indicated the production of acid respectively. The present study coincides with the findings of Guarmer, 2003 where in maximum growth with strain MK-2 showed as optimum pH of 5.0 with OD value of 2.2. Similarly, the strain Mk-4 also showed a maximum growth of pH 7.0 with 2.16 was recorded. The study was focused to assess the NaCl tolerance, where in MK-2 exhibited maximum tolerance. The isolate MK-2 tolerated an enhanced saline condition. It was found that the isolated MK-2 showed maximum tolerance at 3% and 4% and it was found to show an optimum growth up to 6.5% of sodium chloride. Simultaneously strain MK-4 exhibited maximum tolerance at 4% and it was found to show an optimum growth up to 6.5% of sodium chloride. Resistance to bile salt of the isolates could be attributed to their ability to produce bile hydrolase. Bile salt hydrolase (BSH) protects the cells that produce it from the toxicity of conjugated bile salts by deconjugating the bile acids Kostinek, *et al*., 2005. Further study extended in order to elucidate the efficacy of the lactobacillus sp was tested against the different concentration of oxbile. The ability of the strains (MK-2 and MK-4) were able to survive in bile salt is a very important probiotic. The test organismexhibited the resistance against different concentration of ox bile. The isolated *Lactobacillus sp* was able to survive in 0%, 0.3%, 0.5%, and 1% bile salt. The isolated *Lactobacillussp* (MK-2) was also able to multiply in the above mentioned concentrations of bile salt. The strain MK-4 showed maximum survivability in the bile salt which again proved their probiotic ability. Partial purification of bacteriocin was performed by precipitation of cell free supernatant with 70% ammonium sulphate. Precipitation was found to increase the level of bacteriocin. The *Lactobacillussp* (MK-2) mainly responsible to produce bacteriocin.

It was produced highest amount of bacteriocin. The MK-4 strains were bacteriocin was produced lower level of bacteriocin. The bacteriocin produced by them showed strong activity against food borne pathogens Listeria monocytogenes.Todorov and Dicks, 2009 suggested that bacteriocin production was strongly dependent on pH, nutrients source and temperature. Maximum bacteriocin activity was noted at pH 6.0, temperature 30^{o}C and 1.5% NaCl (Naidu and Bidlack, 1999). The amount of protein present in the given sample (MK-2 and MK-4) was quantitatively estimated by Lowry'smethod using bovine serum albumin as standard. The amount of protein present in the given sample was (MK-2) found to be 2.01 mg protein/ml and (MK-4)found to be 1.97 mg protein/ml respectively. Our results were in total agreementwith the work of (Todorov, *et al*., 2004).The molecular weight of the protein was determined by SDS-PAGE gel electrophoresis. The both sample (MK-2 and MK-4) revealed a protein band was recorded, when stained with coo massive blue and it clearly indicated the purity of the protein. The molecular weight of the partially purified protein was calculated compared with protein marker Mk-2 and MK-4responsively. Raffter, *et al*., (2003) studied the HPLC analysis to determination of individual folate forms and are less time-consuming. A number of HPLC methods for folates in various food matrices have been reported recently (Bagley, *et al*., 2006).

Conclusion

Folic acid was live microbial food supplement beneficially affect the host by improving the intestinal microbial balance. Lactic acid bacteria are considered as probiotic bacteria in which *Lactobacillus* and *Bacillus cereus* is a major role. Though *Lactobacillus* is rich in fermented milk, it can be easily isolated from the raw milk sample. Folates are a group of naturally occurring B vitamins of great nutritional importance related to pteroylmonoglutamic acid (folic acid). They exhibit biological activity like folic acid and usually exist in the poly glutamate form typically with five to seven molecules of glutamic acid.

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