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ISOLATION OF LACTIC ACID BACTERIA (PROBIOTIC) FROM HOMEMADE FERMENTED FOOD SAMPLE FOR YOGHURT FORMATION

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INTRODUCTION

The production and consumption of traditional fermented food products becomeing reasingly relevant in the face of rapidly increasing population and food insecurity, more research and development to ensure the safety and nutritional quality of these fermented products is warranted. In addition to preservation, fermented foods can also have the added benefits of enhancing flavor, increased digestibility, improving nutritional and pharmacological values. Lactic acid bacteria have contributed in increased volume of fermented foods worldwide especially in foods containing probiotics or health promoting bacteria.

"Probiotics are live microbial, dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and metabolic activity of the flora of the gastrointestinal tract"(Jeyaram *et al*., 2009).

The word "probiotic" was derived from the Greek word which means "on behalf of". The concept was Lilly and Stillwell (1965) and was intended stimulate substances produced by one microorganism to enhance the growth of another. The word probiotic was used later to refer to animal feed supplements. Probiotics also defined as "selected viable microorganisms use as a dietary supplements having potential for improving health or a nutrition of man or animal following ingestion". The primary probiotic bacteria associated with dairy products have been Lactobacillus acidophilus, L.casei, L.crisparis, L.gasserei, L.johansonni, L.plantarum, L.ruterii, and bifidobacteria etc. Milk or milk products and excellent carrier for these probiotic organisms. Most of them can radily utilize lactose as an energy source for growth. et., al2009).

Lactic acid bacteria (LAB) are one of the microorganisms that dominate fermented food (Guasch-Jane et al., 2005 Robert, 2008). Today, LAB is of essential importance for their role in

most industries of fermented foods as starter cultures. Various metabolic and enzymatic activities of LAB lead to production of volatile substances, which contribute to flavor, aroma and texture developments (Kleerebezemab,2000).Certain LAB strain characterized by their ability to transform lactose and improves the digestibility of fermented dairy products (Weinberg et al.,2007) as well as their preservation (Abdelbasset,2008). They also employed for improvement of the taste, texture and viscosity in the manufacture of dairy products (Soukoulis et al., 2007). The ability of LAB to produce probiotic(Temmer manetal. 2002) and stimulation of the immune system (Kalliomakietal.2001) render this group of microorganisms essential importance in dairy industry.

The Lactic acid bacteria (LAB), generally considered as food grade organisms, show special promise for selection and implementation as protective cultures. There are many potential applications of protective cultures in various food systems (Holzapfel *et al*., 1995). These organisms have been isolated from grains, dairy products and fermenting vegetables. LAB were isolated from vegetables and traditional fermented foods including dhokla, idli, dahi, jalebi, yogurt and cabbage (Patel *et al*., 2012 and Neha *et al*., 2014), dosa (Appam) batter and vegetable pickle (Vijai Pal et al.,2005).The Indian population depends heavily on fermented cereal and legume grains which gives higher digestible protein content, and provides a better balanced ratio of amino acids. Cereals such as rice (*Oryzasativum*), ragi (*Eleusine coracana*), wheat (*Triticum* spp.), barley (*Hordeum vulgare*), and pulses are predominantly used in the preparation of significant number of fermented foods since ancient times. They are very rich source of water soluble fibers like ß-glucan, Galacto oligosaccharides and fructo oligosaccharides, which act as prebiotics and digested by selective groups of LAB (Swennen *et al*., 2006). Traditional Indian fermented product separated by fermentation of milk by lactic acid bacteria. Intake of Dahi has been found to cure diarrhea (Agarwal and Bhasin 2002);and reported to have anti-cholesterolemic(Sinha and Sinha 2000), anticarcinogenic (Arvind *et al*., 2010) and anti-diabetic effects (Yadav *et al*., 2007) and few other benefits (Prajapati *et al*., 2014). The lactic acid

fermentation of vegetables, applied as a bio preservation method to enhance their storage life (Kingston *et al*., 2010).

Considering the rich microbial diversity in such fermented food, and the nutritional and therapeutic potential of the associated microflora, an attempt is made to isolate novel strains and characterize them for their application in functional foods. Lactic acid bacteria (LAB) are gram-positive, acid tolerant and non-spore forming cocci and rods. They are heterogenous group of bacteria comprising about 20 genera within the Phylum Firmicutes. From practical point of view the genera *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc. Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, vagococcus* and *Weisella* have been considered as the principal LAB (Korhonen 2010). Based on end products of glucose metabolism, LAB can be devided into two groups, namely homo fermentative and hetero fermentative (Jay 2000).

Yoghurt is a traditional fermented, milk product popular in India and other Asian countries like Pakistan and Bangladesh. It contains a mixed culture of lactic Streptococcus and Lactobacillus in addition to the well known yoghurt organisms such as S.thermophiles and L.delbrueckii spp. bulgaricus (Masud, T 1991, Raquib M., et AL.2003). Bacterial species currently used in the dairy industry belong to the genera Lactobacillus and Strepococcus. Lactic acidobacteria (LAB) secrete lactic acid as the major, end product of sugar fermentation (Axelsson et al., 1998).Therefore, the isolation and identification of new strains from the indigenous fermented milk products is necessary in order to bring novel strains to the industry. The quality of yogurt available in the market or prepared in the household is however subjected to considerable microorganisms which may cause various defects like gassiness, off flavors and proteolysis in the product. Other factors which influence the quality of yogurt are purity and activity of the cultures, relative proportion of different components of mixed cultures, variations in the incubation temperature, contaminants like spore forming bacteria, yeast, fungi and other pathogens gaining entry in to the yogurt from various sources such as utensils, hands of person and atmosphere of the kitchen. Under laboratory conditions all these factors could be controlled

and selected of lactic acid bacteria of known characteristics could be use to obtain yogurt of consistently uniform quality having the desired acidity and flavor. As no such study has been carried out on isolation of lactic acid bacteria from homemade fermented food sample for the preparation of yogurt under low temperature is around 20˚C-30˚C. Under low temperature conditions it is difficult to make fermented yogurt and therefore there is need to isolate suitable lactic cultures capable setting the yogurt at temperatures like 5˚C, 15˚C and 22˚C.There are several advantages in developing a methods in preparing yogurt at low temperature, since the contaminants are mostly mesophilic, will not be able to grow along with lactic starter cultures. Even yeast which is the major contaminating micro-flora will not grow to the extent in yogurt prepared at low temperatures. A method for the preparation of yogurt low temperature has been standardized using a lactic culture isolate. The yogurt sets at 5˚C, 15˚C, and 22˚μC with mild acidity. The yogurt can be stored or a long time without significant increase in the acidity. The yogurt can be prepared in the household conditions using this culture in winter season as well as in cold regions. A simple method of preparation of good quality yogurt at room temperature would benefit the consumers living in cold regions in preparing and consuming quality yoghurt with characteristic flavoured due to diacetyl production(Javid et al).

Morphology and Biochemistry of:

Lactic Acid Bacteria: They are gram negative, facultative anaerobe, Non spore forming bacteria, grow under anaerobic conditions and cocci or rods which produce lactic acid as the measure and product during fermentation of carbohydrates(Axelsson 2004)

Phylum – Firmicutes

Class – Bacilli

Order – Lactobacillales

Fmily – Lactobacillaceae

Streptococcus: Streptococcus from Greek twisted berry.They are group in chains, string of beads. It is S.salivarius subspp.thermophilus streptococcus. They are gram positive, homo

fermentative,facultative anaerobes belongs to viriadans group. It play main role in yogurt formation.

Phylum – Fermicutes

Class- Bacilli

Order – Lactobacillales

Family – Streptococcaceae

Genus - Streptococcus

Materials and Methods

- Sample collection: Food samples Idli, Jalebi, Curd, Dhokla, Pickle, Whey were collected from homemade preparation in sterile glass bottles and brought to laboratory. The samples were kept at 4˚C and processed within two hours.
- Isolation: Each samples 10 % (w/v) were homogenized in equal volume of sterile milk, here milk is use broth and pour on plates De Man, Rogosa and Sharpe agar (MRS agar, Hi media) were incubated a37°C for 24 to 48 hrs in incubator.
- Identification: Selected isolated colonies were observed and identified as L.bulgarius and S.thermophilus on the baisis of their different morphological characteristics were purified by streak plate method and then preserved at -20°C on MRS agar slants, in MRS slants, in MRS broth containing 10 % glycerol (w/v) and in freeze-dried form. All the cultures were routinely sub cultured at regular intervals and were activated in MRS broth before being used in the experiment(Thakkar *et al*., 2015).
- Milk fermentation: Milk fermentation abilities of LAB isolates were assayed by inoculating litmus milk (10% sterilized skim milk) with 1% active cultures. Observations were made regarding acidification, reduction, coagulation, whey separation and gas production at the end of 24 h incubation at 37°C.
-

Morphological characterisiation of LAB and Streptotococcus thermophilus :

Biochemical tests :

Gram Staining: For gram staining the fixed smear was treated with crystal violets solution for 1 min, this was gently rinsed off and iodine solution applied for 1 min. Ethyl alcohol (95%) was then applied 10 -12 drops to decolorize the strain. Finally Saffarnine was used as a counter stain for 10 second.Then slide was gently rinsed off with water and blotted off. The result was recorded as gram positive and gram negative.

Motility Test: The middle of the slide. Lower a clean cover slip the drop as thought it were hinged at one side avoiding in motility test take a clean, scratch free glass slide. Lable the slide then place 15-20µ lit of the culture in bubbles after that examine the preparation under microscope then the result recorded as motile and non motile.

Protease activity: Plates were prepared with standard plate count agar supplemented with 10 reconstituted sterilized skim milk. The isolates grown in MRS broth were spot inoculated (10 µl) on the agar plates and incubated for 48 h at 37ºC (Taheri et al., 2009).

Amylase activity: Amylase activity was determined by spot inoculating culture of LAB on starch agar plates and incubated at 30ºC for 48 h. Clear zone of amylase activity was detected after flooding the plates with Lugol's solution (Thakkar et al., 2015).

Lipase activity: For the detection of lipase activity, the MRS broth containing olive oil (1%) and Arabicgum (1%) was used to subculture the strains and the activity was detected by using a medium that consisted of tryptone (0.1%), yeast extract (0.5%), NaCl (0.05%), olive oil (0.1, 0.5, or 1%), Arabic gum (1%), and agar (1.5%). Lipase positive colonies were detected by the presence of zone of precipitates around the colonies (Thakkar et al., 2009).

Sugar Fermentation Test: MRS broth containing different Sugars (HiMedia, Mumbai, India) and phenol red as pH indicator was inoculated with active cultures at 1%, incubated anaerobically at 37°C for 24 h. The cultures were identified as positive or negative based on acid and gas production after the incubation period (Thakkar *et al*., 2015).

Oxidase Test: Placed 1-2drops of 1% oxidase reagent on 6cm sq.piece of whaltman filter paper. Transfered a small colony of test organism using a loop onto soaked filter and observed for purple colour develpoment. A negative test does not purple colour.

Catalase Test:Take a drop of reagent on a clean slide an transferred colonies into it and observed the effervescence formation. No effervescence produced.

Indole Test: Tryptopan broth was prepared accourding to the given composition.The broth was dispensed into the tubes and sterlized.The test organisms were inoculated into the tubes and one was as control .The tubes were inoculated at 37˚C for 24-48hrs. After incubation 1ml of kovac's reagent was added to all the tubes including control.The tubes were shaken gently and to stand 1-2min.The tube were observed for the result.

Methyl Red Test: Glucose phosohate broth was prepared accourding to the given composition. The broth was dispensed into the tubes and sterlized. The test organisms (test culture) for 24hrs. After incubation 5drops of methyl red indicator is added the colour changed into the red. It shows positive.

Voges–proskaer Test: Glouse phosohate broth was preparing accourding to three composition .The broth was dispensed into the tubes and sterlized. The test culture were inoculated into the tubes and one was left un inoculated as control. The tubes were incubated at 37˚C for 23-48hrs. After incubation 3ml of alfa naphthol then 40 % potassium hydroxide (KOH) added and mixed properly the pink colour creamson red were observed.

Citrate Test: Citrate broth was prepared to the given composition .The broth was dispensed into the tubes and one was left un inoculated as control. The tubes were incubated at 37˚C for 24-48hrs. After incubation observed turbidity against the control tube turbidity shows positive test.

Antibiotic Sensitivity Test: After 24hrs, the zones of inhibition were observed and compared with the standard sample. It was found that the antibiotic produced by isolates was more effective for all antibiotics and isolate W2- is more effective for Ampicillin antibiotic than other antibiotics.

RESULT AND DICUSSION

Lactic acid bacteria are widely distributed in the nature. In this group are include representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc. They

could be isolated from soils, waters, plants, silages, waste products, and also from the intestinal tract of animals and humans. The lactic acid fermentation, which these bacteria perform has long been known and applied by the humans for making different foodstuffs. It plays an essential role in the production of dairy products and is involved in the production of many other foods and drinks-yogurt sausages, pickles etc. Since decades by these processes the application of well studied starter cultures was established .They should possess stable fermentative characteristics and should be resistant to bacteriophages. In our country at conditions dairy products are prepared from cow, but also from sheep, goat's and buffalo-cow' milk –such as cheese. curds ,yoghurt, yellow cheese, etc.By these products the species composition of lactic acid bacteria is more various and inconstant when compared with those of the trade products. The "wild" strains, in biotechnological aspect are prespectives bcteriocin producer and probiotics. They have a potential use for the establishing of new, so-called "functional foods". The aim of the recent study was the isolation of LAB from homemade fermented food samples for the yoghurt formation. The species belongs to group of the thermophiclic lactobcilli, obligate. Homo fermentative type and is included in starter cultures for the production of yoghurt. The other three lactic acid cultures were reffered to genus Lactobacillus. LAB divided into two groups based on the products from the fermentation of glucose.

Homofermentative lactic acid bacteria and heterofermentative organism.LAB is generally associated with habitat rich in nutrients such as yoghurt, milk, cheese, meat, beverages and vegetables. They could be also isolated from fermented food, soil, milk, lakes, intestinal tract of animals and humans (Tserovkaet at.,2002; Chen et al.,2005;Schillinger and Luke,1989).LAB are widely used as starter cultures in the manufacture of fermented products including milk products such as yoghurt (Axelsson,2004).Many recent studies have shown the health effects of various strains of lactic acid bacteria in humans and animals have tried to describe their action mechanism in the digestive tract. A number and a variety of potential beneficial effects have been published. Some of these effects have already been describe such as the improvement of lactose digestion and the treatment of diarrheal disorders(Gillilard,1990;Drouault and

Corthier,2001).LAB can act s cell factories for production of food additive and aroma compounds and they have a potential use for these establishing the so called functional food(Halzapfel et al.,2001).The samples used in this study are traditional fermented yoghurt, left at room temperature until it tastes sour.They are gram positive, non spore forming rods, catalase negative, microaerophilic, with fermentative metbolizing for their identification to speciehological, cultural, physiological and biochemical characteristics were tested.

MRS media are the best suitable media for the isolation of lactic acid bacteria. After growing the cultures were diluted and tested for purity on the suitable agar medium. By isolating and identifying colonies from these and by doing different biochemical reactions and confirmations are obtained which are described in this Probiotics exact opposite of antibiotic. While doing yoghurt formation certain conditions temperatures and pH are maintained.

Morphological characteristics of microorganisms isolated from samples:

Samples	I 1*	I 1	I 2*	I 2	I 3*
Size	Moderate	Small	Small	Moderate	Moderate
Shape	Circular	punctiform	Circular	Circular	Circular
Colour	Buff white	white	Pale yellow	Buff white	Buff white
Margin	Entire	Entire	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth	Smooth	Smooth
Elevation	Raised	Flat	Flat	Raised	Raised
Consistancy	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid
Opacity	Transucent	Opeque	Transucent	Transucent	Transucent
Motility	Motile	Motile	Motile	Motile	Motile
Grams Nature	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters

I 3	C 1	C2	C3*	D3	D4
Small	Small	Small	Small	Moderate	Small

Punctiform	Circular	Punctiform	Punctiform	Circular	Punctiform
Buff white	Pale yellow	Buff white	Buff white	Pale yellow	Pale yellow
Entire	Entire	Entire	Entire	Entire	Entire
Smooth	Rough	Rough	Smooth	Smooth	Smooth`
Raised	Flat	Flat	Flat	Smooth	Smooth
Mucoid	Butyrous	Butyrous	Mucoid	Butyrous	Mucoid
Transucent	Opeque	Opeque	Opeque	Transucent	Transucent
Motile	Motile	Motile	Motile	Motile	Motile
Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters	Gram +ve Cocobacilli in clusters

smaples	P1*	P1	P2*	P2	P3
Size	Large	Large	Small	Small	Small
Shape	Rhizoid	Rhizoid	Elongated	Punctiform	Punctiform
Colour	yellow	Yellow	Yellow	Yellow	Yellow
Margin	Undulate	Undulate	Flat	Entire	Entire
Surface	Smooth	Smooth	Smooth	Rough	Smooth
Elevation	Raised	Raised	Flat	Flat	Raised
Consistency	Mucoid	Butyrous	Butyrous	Butyrous	Butyrous

Opacity	Translucent	Translucent	Translucent	Translucent	Translucent
Motality	Motile	Motile	Motile	Motile	Motile
Grams nature	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated

W2*	W2	W1	J1*	J3	J3*
Small	Small	Small	Small	Small	Small
Punctiform	Punctiform	Punctiform	Punctiform	Circular	Punctiform
Buff white	Buff white	Buff white	Buff white	Buff white	Buff white
Entire	Entire	Entire	Entire	Entire	Entire
Smooth	Smooth	Smooth	Rough	Smooth	Smooth
Conver	Raised	Raised	Flat	Flat	Flat
Mucoid	Mucoid	Mucoid	Butyrous	Translucent	Mucoid
Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
Motile	Motile	Motile	Motile	Motile	Motile
Gram+ve cocobacilli in clusters	Gram+ve cocobacilli in clusters	Gram+ve cocobacilli in clusters	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated	Gram+ ve diplo bacilli in isolated

I-Idli, C-Curd, D-Dhokla ,P-Pickle, W-Whey, J-Jalebi

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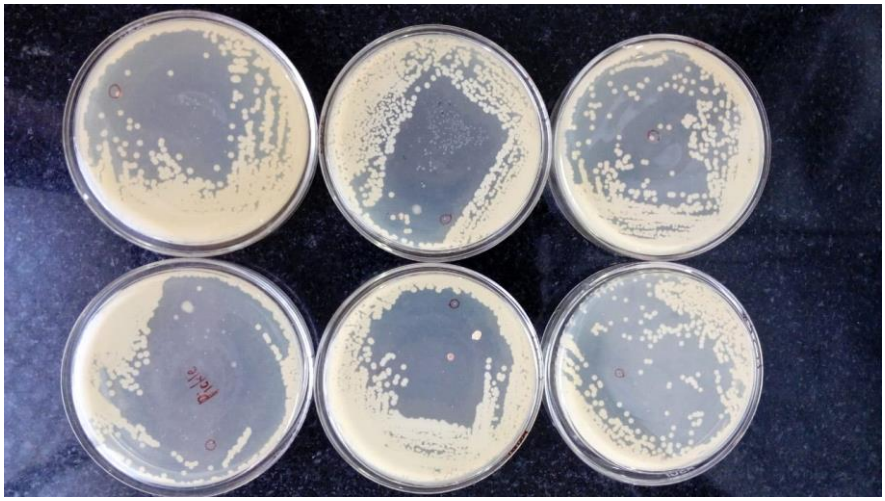
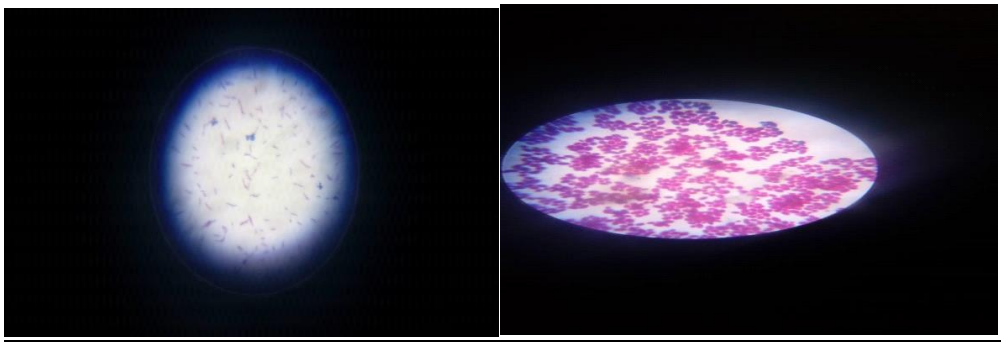


Streak plate method



Bacilli (gram positive) cocci (gram positive)

Sugar Fermentation Test Methyl Red Test

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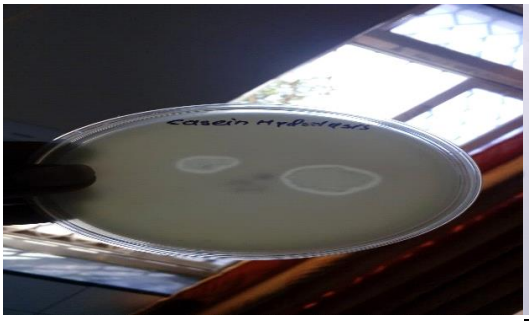

Casein Hydrolysis

Starch Hydrolysis

Antibiotic Sensitivity Test

Name initials given to plate (M-Manisha)

Plane yogurt

Fruit Yogurt

Yoghurt production

Homogenised whole / low fat milk

Heat at 80-90^{o} c for 30-60 minutes

Cooled to 40-45^{o} c

Culture added (2%) at 40-45^{o} c

Incubated at 43^{o}c for 3-7 days

Fermentation stopped by

Addition of fruits

Packaging

Storage at 4^{o} c

CONCLUSION

Cultural, morphological, physiological and biochemical characteristics showed that the following genera and species of LAB and probiotic bacteria were present in the dairy products examined Lactobacillus delbrueckii, Lactobcillus acidophilus(YD). From the current investigation it can be concluded that the LAB and S.thermophilous is use for the production rich quality yoghurt formation. The media components can be manipulated to obtain the maximum growth from these culture. From the above project review it can be concluded that

LAB (PROBIOTICS) isolated from the homemade fermented food samples are useful for the yoghurt preparations was carried out successfully.

FUTURE PRESPECTIVE

- To check whether the ingredients in yogurt can be change using the different types of chepest fruits available in the market.
- To vary other parameters of production process.
- To go for production on large scale.
- To vary the fermentation time period.

APPLICATION OF PROBIOTICS

Lactic acid bacteria widely used in the manufacture of yoghurt. Some of these beneficial lactic acid consumption include:

1. Improving intestinal tract health.

2. Enhancing the immune system synthesizing and enhancing bioavailability of nutrients.

3. Reducing symptoms of lactose tolerance decresing prevalence of allergy in succiptible individuals.

4. Reducing risk of certain cancers.

5. Yogurt containing bacteria L.bulgaricus and S.thermophilus improve lactose digestion and eliminate symptoms of lactose toler. The bacterial effects are also due to microbial beta-galactosidase in fermented milk product.

6. Yogurt has an adequate amount of nutrients to improve health of consumer. Yogurt is an excellent of protein, calcium, phosphorous, riboflavin(vit B12),thiamine (B1) & vit B12 valuable source of folate, magnesim and zinc.

7. Probiotics can be generalize under the following categories: maintenance of normal intestinal microflora balance in infant old age ,improvement of lactose tolerance and digestibility of milk products. Anti tumerogenic acticity, reduction of serum cholesterol.

8. Potential use in the development of new fermented and probiotic foods, and other applications.

REFERENCES

- M.Lind, "Natural Therapy: Using Yogurt to treat Fungal Infections," 2009 http://voices.yahoo.com/natural-thera-py-yogurt-treat-fungal-infections-32648848.html?cat=68
- T.Vasiljevic and N.P.Shah, "Prbiotics-From Metchnikoff to Bioactives,"International Dairy Journal,Vol.18,No.7,2008,pp.714-728.doi:10.1016/j.idairyj.2008.03.004 4.conclusion
- N.P.Shah, "Functional Xultures and Health Benefits," International Dairy Journal, Vol.17,No.11,2007,pp.1262-1277.doi:101016/j.idairyj.2007.01/014
- F.Guarner,B.Koletzko,L.morelli,S.Salminen,G.Perdigon and g.Corthier, "ShoulD Yogurt Cultures Be Con-sidered Probiotic?" BRITISH Journal of Nutrition,vol.93,No.6,2005,pp.783-786.doi:10.1079/BJN20051428
- S. Parvez,K.A Malik .S.A.H. Kang and H.Y Kim, "Probiotics and Their Fermented Food Products Are Benficial For Health ," Journal of Applied Microbiology, Vol. 100, No.6,2006.pp.1171-1185. Doi:10.1111/j.1365-2672.2006.02963.X
- M.D. Vrese, C. Laue,J.Schrezenmeir, S. Fenselau, A.Stegemann and B.Richter, "Probiotics: Compensation for Lactase Insuffciency," American Journal of Clinical Nutrition, Vol.73, No 2,2001,pp.421-429.
- M.C.Mckinley, "The Nutrition and Health Benefits of Intnol y, Vol.58,No.1, 2005,pp.1.12 doi: 10.1111/j.1471-0307.2005.00180.x Yoghurt," ernational Journal of Dairy Tech.Abdelgdir, W.S., H.S Hamad, P.L. Moller and M.

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