INVITRO EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF PROBIOTIC BACTERIA FROM CURD

J.FATHIMA[1], J.Ishwarya[2], S.Agila[3]

1 Research scholar , PG and Research Department of Microbiology, Kamban college of Arts and Science for Women, Tiruvannamalai – 606603.
2&3 Assistant Professor , PG and Research Department of Microbiology, Kamban college of Arts and Science for Women, Tiruvannamalai – 606603.

ABSTRACT

Curd is a semisolid sourish food prepared from fermented milk which is richest source of "probiotics". The investigation carried to prove the antimicrobial and antioxidant activity of probiotic bacteria from curd. It can be used as a preservatives in food products also it proved as a best surviving in the low pH and bile conditions. This work was to prove that the *Lactococcus* can produce antimicrobial substance which can inhibit the growth of pathogenic bacteria.

INTRODUCTION

Lactic acid bacteria (LAB) are Gram positive coccoid or rods, non-aerobic but aero-tolerant, able to ferment carbohydrates for their energy and lactic acid production. **(Caprice and Fitzgerald 1999)**. These microorganisms are found in milk, meat, fermented products, fermented vegetables and beverages. Lactic acid bacteria inhibit the growth of pathogenic and deteriorating microorganisms, maintaining the nutritive quality and improving the shelf life of foods. They have also been used to improve flavor and texture of various foods.

Lactic acid bacteria includes various major genera like *Lactobacillus, Lactococcus, Corynebacterium, Enterococcus, Lactosphaera, Leucconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella*. Other genera are *Aerococcus, Microbacterium, Lactobacillus acidophilus, L. planterum, L. casei, L. fermentum, L. reuteri, Lactococcus lactiscremoris* some most common species **(Parada *et al.*, 2003)**.

SCIENTIFIC CLASSIFICATION

KINGDOM : Bacteria
DIVISION : Firmicutes
CLASS : Bacilli **ORDER** : lactobacillales
FAMILY : Streptococcaceae
GENUS : L*actococcus*
SPECIES : *lactis*

L.lactis is of crucial importance for manufacturing dairy products, especially butter milk and cheese, meat products, poultry, aquatic products, canned food, fruit juices, plant proteins, backing foods, fast foods, beverage drinks, medicine and health care products **(Sirisansanegakul*et al.*, 2007).** Like can be preserved by the vegetables Tomato, Potato, Cucumber, Beans, Carrot and fruits like Grapes, Strawberry, Apple, Orange.

Probiotics are microorganism's active factors, which show the beneficial impacts on the host health. Probiotics significantly affect the bioavailability of nutrients in the human body by facilitating the absorption of magnesium and calcium from milk proteins, digesting lactose and producing folate and B vitamins. Lactobacillus and Enterococcus species are common lactic acid bacteria (Gram-positive and non-toxic bacteria), which are usually consumed as probiotics. Lactic acid bacteria (LAB) are generally recognized as safe microorganisms. Most of probiotics include LAB group, which is isolated from safe sources, such as fermented dairy products and can be introduced as probiotics.

Lactic acid bacteria (LAB) have been extensively studied for their commercial potential, food preservation and health benefits. They are industrially important microorganisms used worldwide mainly

in the dairy industry for manufacturing fermented milk products and cheese.

Lactic acid bacteria and their fermented products are thought to have health promoting probiotic effects in human such as inhibition of pathogenic organism, antimutagenic and reduction of blood cholesterol. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on host (the Food and Agriculture Organization/World Health Organization (FAO/WHO). Most probiotics commercially available today belong to the genera *Lactobacillus* and *Bifidobacterium*. Lactic acid bacteria for use as a probiotic culture must be tolerant to gastric acid and bile, which enables selected strains to survive, grow and perform its therapeutic benefits in the intestinal tract.

The term probiotic, literally meaning "for life", was first addressed by **(Lilly and Stillwell 1965)** and was used to describe substances produced by protozoa to stimulate yeasts that, when ingested, are able to reach the intestines in sufficient numbers to confer health benefits to the host **(Schrezenmeir and De Vrese, 2001)**.

Milk proteins are an abundant source of bioactive peptides. Milk and especially its fermented products in recent time are gaining in popularity as an component in health beneficial functional types of food, which can have a preventative effect on some modernage diseases caused by improper diet such as cardiovascular diseases, diabetes type II and obesity. These peptides are inert in the initial protein of the milk (casein and albumins), and can be activated and released into the medium by several processes such as enzymatic hydrolysis of the protein by digestive enzymes, fermentation of the milk by various microbiological strains and proteolysis by vegetative or microbiological enzymes in cheese production **(Hannu)**.

The fermentation of the milk with lactic acid bacteria results in delivering a vast number of bioactive peptides and free amino acids with different biological activity such as inhibition of the angiotensin converting enzyme **(Nielsen *et al.*, Tomovska *et al.*,) , immune activity (Coste *et al.*,)and antioxidative activity (Pena-Ramos *et al.*,)**.

Antioxidant vitamins in milk have a great contribution in the daily intake of these nutrients. Vitamin E and carotenoids, for example, are located in the membranes of the fat lobules in milk where they prevent auto oxidation of the milk fat. Vitamin C is an important antioxidant which has a complex interaction with the iron and is an electron donor in the conversion of the tocopheroxyl radical back to antioxidant active vitamin E **(Lindmark and Akesson)**.

Antioxidants can serve as preventative agents from different types of diseases such as cancer, atherosclerosis and diabetes. Therefore consumption of natural antioxidants through food is helpful for the human health. The fermentation of milk by lactic acid bacteria releases a large number of peptides and amino acids with biological actions, such as angiotensin converting enzyme inhibitory, immune modulatory, opioid and antioxidantactivities.

Curd is a semisolid sourish food prepared from fermented milk, which can be eaten plain or with addition of salt/pepper/sugar/fruits. Its origin is linked to India, where it is consumed throughout the year. The word da-hi seems to be derived from the Sanskrit word dahi, one of the five elixirs (panchamrita) often used in Hindu rituals. Curd (Indian dahi) is popular due to its favorable impact on health and high nutritional value. It has been designated as functional food, which boosts natural as well as acquired immunity and improves stamina. Curd forms the richest source of "probiotics". Probiotics contain living microorganisms (good bacteria), which upon ingestion exert health benefits beyond inherent general nutrition. At the beginning of 20th century, Elie Metchnikoff showed that the consumption of yoghurt (fermented milk) products resulted in sound health and enhanced life-span of Bulgarian peasants.[1] Moreover, the individuals, who consumed local yogurt were able to protect their intestines from the destructive effects of other pathogenic bacteria such as *Lactobacillus, Bifidobacterium, Escherichia Coli, Enterococcus, Bacillus, Streptococcus and some* fungal *Saccharomycesstrains.*

MECHANISM OF ACTION

Curd forms the richest source of "probiotics". Probiotics contain living microorganisms (good bacteria), which upon ingestion exert health benefits beyond inherent general nutrition. Presently, there is growing awareness among people for the nutritional control of ailments. Nutritional supplements like Curd form natural healing foods. These probiotics offer beneficial and healthy micro flora to the alimentary canal through diet without any risk of adverse effects. Lactic acid bacteria (LAB) e.g. *lactobacilli* is one such example of advantageous microorganisms. Probiotics promote the health of the host by boosting the immune system of the body. Natural immunity is strengthened by the curd through

stimulation of mucosal and systemic host immunity, which is manifested through enhanced level so immunoglobulins, activated macrophages, high levels of cytokines and natural killer (NK) cell activities in the patient. Further, probiotic bacteria can trigger a cascade of immunological defense mechanisms by binding to recognition receptors, such as Toll-like receptors (TLRs) expressed on the surface of epithelial cells. Moreover, they may enhance immune status of HIV patients by increasing the production of immune cells, such as CD_4 + T-cells (that mediate and control the balance of pro-inflammatory and anti-inflammatory cytokines and chemokines). Hence, curd may be viewed as the potential remedy while designing clinical studies for the treatment of illnesses like allergy, urinogenital infections, HIV, cancer, *Helicobacter pylori* infection, liver disease, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and pancreatitis. Different probiotic strains generate diverse immune responses dependent on immune system of the host. Non-specific therapeutic modulation of probiotics in the host is mainly due to adherence and colonization of the gut, suppression of growth or epithelial binding/invasion by pathogenic bacteria, production of antimicrobial substances, and improvement of intestinal barrier function.

ROLE OF CURD

IN THE TREATMENT OF AIDS (ACQUIRED IMMUNO DEFICIENCY SYNDROME)

Lactobacillus rhamnosus GR-1 administered in the form of curd/yogurt has shown promising results for the immune function of people living with HIV (Human Immunodeficiency Virus). Moreover, other micronutrients present in curd have also demonstrated the ability to improve immune function and delay disease progression. People infected with HIV often suffer from altered gastrointestinal function, rapid decline of CD lymphocytes (associated with enhanced intestinal permeability), altered gut micro biota profiles, decline of *lactobacilli* and *bifidobacteria,* diarrhea and higher concentration of pathogenic species such as *Candida albicans* and *Pseudomonas aeruginosa*. Scientific investigations have supported the beneficial role of vitamins B, C, E, folic acid, selenium and whey protein, which are all present in curd in the management of HIV. Thus, curd provides a safe, cost effective, and natural approach that creates a barrier against microbial infection. Furthermore, it significantly decreases the infectivity of HIV.

IN THE TREATMENT OF CANCER

Gastrointestinal (GI) tract cancer, Oesophageal cancer, Gastric cancer (GC), *H. pylori* (HP) and Colorectal cancer (CRC) are the third most frequent cancers worldwide (more than 1 million new cases/year) and the fourth most common cause of cancer death, with about 500,000 deaths annually. Good nutrition forms an essential component of GIT cancer treatment. Eating the right kind of food before, during, and after treatment of cancer makes the patient better and stronger. But nutrient needs vary from person to person. Therefore, eating a right combination of proteins, carbohydrates, fats, vitamins, and minerals (through curd) help in fighting cancer. Several animal studies confirm that the intake of curd/fermented milk like curd containing probiotic bacteria inhibit tumor formation and proliferation. It has been demonstrated that a 6-week administration of *L. acidophilus* fermented milk supplements like curd resulted in lower concentrations of soluble bile acids in faeces of colon cancer patients, which was responsible for cytotoxic effect on colon epithelium. It has been suggested that lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumor cells in culture and inhibit their growth. In a study, lactic acid bacteria significantly reduced the growth and viability of the cancer cell line HT-29. In another study, milk fermented with *B. infantis, B.bifidum, B. animalis, L.acidophilus* and *L. paracasei* inhibited the probiotics and production of short-chain fatty acids (SCFA- inhibit hepatic cholesterol synthesis). Consuming 200 g/day of yogurt containing *L.acidophilus* L1 contributed to a 2.9 % decrease in serum cholesterol concentration of hypercholesterolemic patients.

IN THE TREATMENT OF LIVER DISEASES

Non-alcoholic Fatty Liver Disease (NAFLD) has been reported to have high prevalent rate world-wide. Causal factors of NAFLD are excessive formation of harmful compounds such as bacterial lipopolysacchrides, inflammation inducing substances and overgrowth of gut micro flora. Medicines such as polymixin B and metronidazole are currently employed to surmount the overpopulation of pathogens in the patients of NAFLD. However, it is not a satisfactory therapy. Therefore, usage of probiotics such as curd is recommended. Bacteria present in the curd have the capacity to modulate micro flora overpopulation. However, most of the studies are confined to animals only. Hence, approving probiotics as a treatment option requires large scale clinical trials. Alcoholic Liver Disease (ALD) - ALD

is a cause of a high rate of morbidity and mortality worldwide. Alcoholic steatohepatitis (ASH) and severe ALD is apparent in approximately 30 % of heavy drinkers. Various *in vivo* studies done on animals and humans have shown the presence of bacteria in the bowel tracts, which produce high level of endotoxin Unfortunately.

IN THE TREATMENT OF INSOMNIA

Being unable to sleep at bed time is commonly referred to as insomnia. The symptoms of insomnia include waking up during the night; feeling restless in the morning, fatigue, irritability, depression, anxiety, difficulty in concentrating, headache and digestive problems. One can effectively treat occasional insomnia at home with dietary supplements, including eating yogurt before bed-time. Curd contains several nutrients with potential sleep-promoting properties. Tryptophan is a primary amino acid of the body to produce serotonin and melatonin. Both of these brain neurotransmitters are involved in inducing sleep and relaxation. Group B vitamins are also needed in the synthesis of serotonin. Deficiencies of group B vitamins and minerals may disturb sleep. It has been shown that preparations containing melatonin, magnesium, and zinc taken daily for 8 weeks, 1 hour before bed-time improved the sleep quality. Eating foods rich in tryptophan, such as curd, facilitate the synthesis of serotonin and melatonin thereby, inducing sleep. People experiencing discomfort with milk due to lactose intolerance may be able to tolerate curd without experiencing any discomfort because most of the varieties of curd contain less lactose. The component so curd that are beneficial in ameliorating insomnia include: Calcium, which reduces wakefulness and restlessness. Magnesium, which enhances the secretion of melatonin from the pineal gland, prevents nervousness and induces sleep. Vitamin B_{12} (cobalamin) prevents confusion, dementia and fatigue. Vitamin B_5 (pantothenic acid) is good for relieving stress and anxiety and Folic Acid promotes hemoglobin synthesis and enhances alertness.

IN THE TREATMENT OF DIABETES AND IMPROVEMENT OF LIPID PROFILE

Type 2 diabetes mellitus (T2DM) has rapidly increased worldwide during the past few decades. It is usually accompanied by an increased production of free radicals and impaired anti-oxidant defenses. Anti-oxidative mechanisms of probiotics (containing lactic acid bacteria) could be assigned to reactive oxygen species scavenging, metal ion chelation, enzyme inhibition and inhibition of ascorbate autoxidation. Animal studies have further confirmed that *Lactobacillus acidophilus* and *Lactobacillus casei* attenuate oxidative stress and have antidiabetic effect. Modification of gut micro flora by probiotics may be seen as a novel means of regulating glucose metabolism and improving the condition of T2DM. Dyslipidemia is the leading cause of cardiovascular diseases (CVD) in type 2 diabetics. Yogurt/curd consumption caused a 4.5 % decrease in total cholesterol (TC) and a 7.5 % decrease in LDL-C compared with the control group. It was shown that probiotic yogurt consumption significantly decreased TC, LDL-C,TC:HDL-C (High-density lipoprotein) and LDL-C:HDL-C ratio compared with the conventional yogurt. Among all probiotic strains investigated for cholesterol- lowering effect,

L. acidophilus has been studied most widely. *In vitro* experiments have shown numerous possible mechanisms, which include de conjugation of bile acids by bile salt hydrolase, assimilation of cholesterol binding to cell walls of appropriate treatment of these patients is not available till date. However, probiotics can be administered to relieve ALD symptoms because they can modulate the gut and immune systems. Excessive growth of gut micro flora, bacterial translocation, and endotoxemia is found in patients with cirrhosis. Probiotic therapy modulates bio-ecological system in the intestinal tract by preventing the growth of pathogens. It also improves the mucosal layer and preserves intestinal epithelia cells. Hepatic Encephalopathy (HE) is an acute as well as chronic liver disease, which occurs in at least 50%to70% of patients with cirrhosis. Ammonia produced by the gut flora is released to the portal system that disrupts the central nervous system producing neuropsychiatric syndrome. Probiotics have reduced the bacterial urease activity, alleviated pH and ammonia absorption and decreased intestinal permeability. These actions are favourable for treating hepatic encephalopathy. But, there are limited clinical trials confirming these facts. Viruses like hepatitis B and C virus (HBV and HCV) are the causative agents leading to long term hepato cellular injury. Increase in plasma levels of endotoxin, high amounts of the pro- inflammatory cytokines and liver necrosis is reported in the patients with HBV and HCV. Few reports suggest that the improvement of endotoxemia could be achieved by increasing *bifidobacteria* and *lactobacillus* numbers. But still a lot of work is needed to establish the usefulness of probiotics in this area.

LAB are group of Gram-positive cocci and rods, catalase negative occurring naturally in variety of niches **(Hammes and Hertel, 2006; Mohania *et al.*, 2008)**. Probiotics are defied as "Live microorganisms when administrated in adequate amounts confer a health benefit on host" (FAO/WHO, 2011). Most probiotics available today belong to genera Lactobacillus and Bifidobacterium. LAB are most important group of microorganisms used in food fermentation, they contribute to the fast and texture of fermented products and inhibit food spoilage and pathogenic bacteria by producing antimicrobial substances (lactic acid, hydrogen peroxide, bacteriocin) **(Phillip *et al.*, 2012)**. Several mechanisms by which probiotics mediates their health benefits on the host have been suggested, and can be divided in to three categories; (i) certain probiotics have antibacterial activity and can exclude or inhibit pathogens; (ii) probiotics bacteria can enhance the intestinal epithelial barrier; (iii) probiotics bacteria are believed to modulate host immune response **(Ezendam and Loveren, 2006; Marco *et al.*, 2006; Lebeer*et al.*, 2008; Lebeer*et al.*, 2010)**. To perform their effect in the intestine probiotics bacteria should be capable of surviving passage through gastro intestinal tract (GIT). Thus it is essential for bacteria to have protection systems to withstand the low pH in the stomach, digestive enzymes and bile of the small intestine **(Cotter and Hill, 2003; Jensen *et al.*, 2012)**. LAB were successfully isolated from curd samples **(Ghosh*et al.*, 2011)**. The aim of this study was to invitro evaluation of antimicrobial and antioxidant activity of probiotic bacteria from curd.

MATERIALS AND METHODOLOGY

COLLECTION OF SAMPLE

The curd sample was collected from various commercial producers available in local markets from the retail shop of Tiruvannamalai for the isolation of *Lactococcus lactis*. Samples were transported into the laboratory for physiochemical parameters determination.

ENUMERATION OF BACTERIA

Total bacterial load in the samples were determines by serial dilution with spread plate technique samples were diluted and aliquots were plated onto plate count agar media. After incubation at 37°C for 24 hours total bacterial load was determined in the form of cfu/ml.

ISOLATION OF LACTOCOCCUS LACTIS

Complex medium (CM), Man Rogos a sharpe (MRS) media and litmus milk agar media used for selective isolation of *Lactobacillus* where as the isolate from curd samples were streaked on three selective media and incubated at 37°C for 24 – 48 hours.

CHARACTERIZATION and IDENTIFICATION OFLACTOCOCCUS LACTIS

With a view to identify the isolates upto species level cultural, morphological andbiochemical characters of the isolates were carried to observe confirm the organism.

DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF CURD

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was evaluated using the method of son andLewis DPPH was used as a stable radical. A volume of 2ml of DPPH in ethanol (50mm) was added to 2ml of the curd sample, mixed vigorously and allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured at 517nm. Ethanol was used as a blank, while DPPH solution in ethanol served as the control. The radical scavenging activity of the sample was expressed as % inhibition of DPPH absorbance.

Inhibition = [(A control – A test) / A control] × 100 Where:

- A control is the absorbance of the control sample (DPPH solution without curd) and

II) A test is the absorbance of test sample (DPPH solution plus curd sample)

SCAVENGING OF HYDROXYL RADICALS

Scavenging activity of hydroxyl radicals by bacteria was measured according to the method of Guo. Overnight bacteria samples with different concentration (12.5, 25, 50 and 100µl) were inoculated into samples containing O – phenanthroline (0.1% w/v) 2.5 mMFes$O_4$and 20mM$H_2\ O_2$. The samples were then incubated for 90 minutes at 37°C. The absorbance samples were read at 536nm, and free radical scavenging activity was determined as follows:

Scavenging activity (%) = [(A_1 -A_2) / (A_1–A_0)] × 100%

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Samples were mixed at various concentrations (12.5, 25, 50 and 100 µl) with Pyrogallic acid (0.05M, 0.1ml) The samples were incubated at 25°C for 30 minutes in the dark. Absorbance sample was measured at 320nm and superoxide radical scavenging was analysed as follows.

Scavenging rate (%) = [1 -A_{320}nm sample - A_{320}nm blank] × 100%

RESISITANCE TO LOW PH

Lactococcus lactis obtained from overnight culture were harvested by centrifugation for 10 minutes at 500rpm and 4°C, washed twice with PBS buffer (pH 7.2) and adjusted to pH 2.5 Resistance were assessed in triplicates in terms of viable colony counts and enumerated on MRS agar after incubation at 37°C for 0,1,2 and 3 hours, indicating the time spent by food in the stomach as described by Maragkoudakisa et al (2006) and Zoumpopoulou et al (2000), also growth was monitored at 620nm **(Bassyouni*et al*., 2012)**.

RESISTANCE TO BILE SALT

The tolerance against bile was carried out based on the intestinal bile concentration 0.3% (w/v) and the staying time of food in small intestine is 4 hours (Kumar and Murugalatha, 2012). MRS medium containing 0.3% (w/v) bile concentration was inoculated with overnight culture. Viable colonies were counted forevery hour of incubation time on MRS agar and also growth was monitored 620nm.

BILE SALT HYDROLYSIS

Bacterial culture were grown overnight in MRS broth at 37°C with 5% C$O_2$and streaked in triplicates on MRS agar containing 0.5% (w/v) taurodeoxycholic acid. The hydrolysis effect was indicated by different colony morphology from the control MRS plates after 48 hours of anaerobic incubation at 37°C.

ANTIBIOTIC SUSCEPTIBILITY TEST

Disc Diffusion method was used to determine the antibiotic susceptibility of the isolated

bacterial strain. After spreading of each bacterial strain in anaerobic condition for 24 hours on puller Hinton agar plates, antibiotic discs (PadtanTeb Co, Tehran, Iran) were placed on the plates using sterilized forceps. The plates were incubated for 24 hours at 37°C. Next, the clear zones around each disc were measured.

ANTIMICROBIAL ASSAY

E coli, Pseudomonas, Salmonella, Bacillus, Staphylococcus bacterial isolates were used for the analysis. *Aspergillus, Fusarium, Penicillium, Mucor, Rhizopus* fungal species were also tested. Bacterial isolates were cultured in suitable selective medium for 18 hours at 37°C. The fungal strains were cultured in SDA for 72 hours at 37°C. The growth of culture was observed and maintained.

PREPARATION OF CELL FREE SUPERNATANT

The *Lactococcus lactis* were cultured individually in MRS broth medium for 24 hours at 37°C. The culture was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was filtered using 0.22µm fitter.

ANTIBACTERIAL SUSCEPTIBILITY ACTIVITY

To determine the antagonistic activity of *Lactococcus* by the disc diffusion method as follows. The discs were prepared with Whatman's filter paper with a diameter

of 6mm. The paper were sterilized and the prepared disc was aseptically soaked in the cell free supernatant. Sterile muller Hinton agar was aspectically poured into sterile petriplates and the overnight culture of test organisms were inoculated. Then, sterile forceps were used to place disc aspectically on the surface of muller hinton agar plates. Streptomycin was used as standard control.

ANTIFUNGAL ACTIVITY

Antifungal activity was evaluated using the agar diffusion method, (Arasu *et al.*,). The bacterial strain of *Lactococcus* was streaked on the plate containing 25ml of MRS agar and plates were incubated for 3days at 37°C. About 50µl of fungal suspension with 10^8CFU/ml was incorporated into SDA medium. It was overlaid on MRS agar with *Lactococcus*. The plates were incubated for 3 days at 37°C and the zone of inhibition was analyzed.

MINIMUM INHIBITORY CENCENTRATION

Minimum Inhibitory Concentration (MIC) of both bacteria and fungi were evaluated separately by the tube dilution method. To determine the MIC of bacteria and fungi, 1ml of cell free extract of *Lactococcus* was serially diluted. All tubes were inoculated with fungi and bacteria separately. The fungal agent ketoconazole was used as standard control. The MIC was analyzed as the lowest quantity of the cell tree extract that completely inhibited the growth of the organisms

RESULT AND DISCUSSION

In the present investigation was made to prove the validity of the probate as a medicine the investigation have been undertaken to evaluate the antimicrobial and antioxidant activity of probiotic bacteria from curd.

Lactococcus a group of Gram positive, non spore forming cocci, and fastidious organism frequently isolated from milk and curd. This naturally occurring bacteria are non pathogenic to human and animals hence "Generally Recognized As Safe" [GRAS] organisms. These bacteria have been receiving considerable attention as "Probiotics" because of their innate ability to exert antagonistic activity against non pathogenic and spoilage organisms. Many of the researches been devoted to isolating probiotic emphasison the health promoting properties with made of antimicrobial action.

In this study, the importance of the selection of probiotic bacteria from traditional fermented food 'curd' that can survive the human gastrointestinal tract and counter healthbenefits is emphasized.

The colony morphology and Gram staining on complex medium shows the white, small round opaque, mucoid shape colonies. Gram staining shows purple coloured cocci in chains it was gram positive cocci in chains. Non motile, non capsulated, non spore forming organisms were Indole negative, methyl red positive, voges proskauer negative, citrate positive, catalase negative and oxidase positive.

The curd sample with *Lactococcus lactis* records its highest value for the antioxidant activity of 56.51% and the lowest value for antioxidant activity 39.43% neutralization of DPPH free radical antioxidant scavenging activity 89% superoxide radical antioxidant scavenging activity and 90% reducing activity.

The *Lactococcus lactis* resistant to low pH was the major selection for probiotic strain. The organism was able to survive at pH 3.0 during three incubation. Nine strains of *Lactobacillus* showed very high resistance to low pH (*L. Plantarum, L. Pentosus, L. Paracasei* Sub Specieus *Paracasei* with final populations exceeding 8 log cfu 1ml, whereas 12 strains showed high resistance to low pH.

The strain resistant to low pH was screened for their ability to tolerate the bile salt *Lactococcus lactis* shows partial bile salt hydrolysis activity.

The antimicrobial activity of the *Lactococcus lactis* with disc diffusion technique. The maximum level of inhibition (19 – 33mm) and minimum level of inhibition (15 – 25mm). Effective *Lactobacillus* isolates from yoghurt sample considered for novel probiotics.

The study was carried out to prove that the *Lactococcus* can produce antimicrobial substance to inhibit the growth of pathogenic bacteria. The furthermore studies needed to improve the works, in the future.

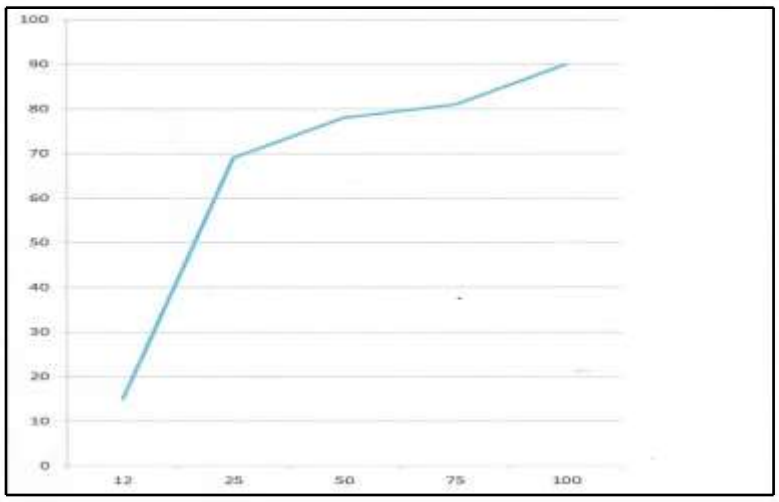
Survival in pH 3.0 – OD620 value.

S.No	Isolates	OD at 620 mm at different time interval(hours)			
		0	1	2	3
1.	*Pseudomonas*	1.1	1.1	1	1.2
2.	*Aspergillus*	0.99	1	0.98	1
3.	*Fusarium*	0.71	0.7	0.7	0.7
4.	*Staphylococcus*	0.56	0.58	0.59	0.58
5.	*Escherichia coli*	0.4	0.38	0.35	0.3

Tolerance against 0.3% bile - OD620 Values

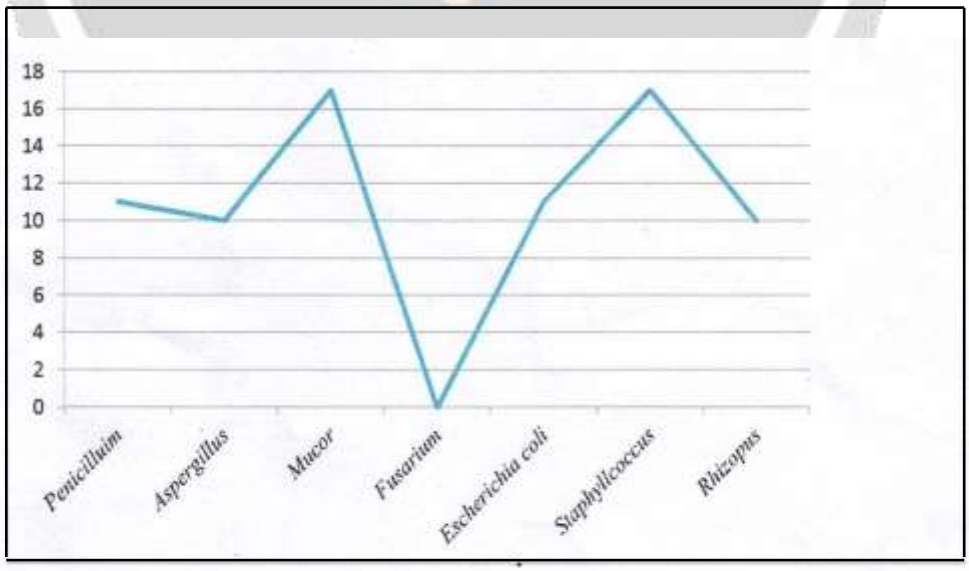
S.No.	Isolates	OD at 620 mm at different time interval (hours)				
		0	1	2	3	4
1.	*Pseudomonas*	0.55	0.042	0.049	0.049	0.07
2.	*Aspergillus*	0.038	0.036	0.037	0.04	0.042
3.	*Fusarium*	0.031	0.03	0.029	0.031	0.032
4.	*Staphylococcus*	0.02	0.023	0.03	0.028	0.031
5.	*Escherichia coli*	0.03	0.028	0.025	0.02	0.018

ANTIOXIDANT ACTIVITY OF CELL FREE EXTRACT ISOLATED FROM LACTOCOCCUS LACTIS



THE INHIBITORY EFFECT OF ISOLATED STRAINS AGAINST PATHOGENIC MICROORGANISMS

CONCLUSION



S (Strong r ≥ 20mm), M(moderate r ≤ 20mm and ≥ 10mm)

The probiotic strains that one isolated from curd have a broad spectrum of antimicrobial activity and antioxidant activity. It can be used as a preservatives in food products also. It proved to be a best surviving in the low pH and bile conditions. It also

possess surface binding properties capable of colonizing the gastro intestinal tract, which is important for antimicrobial activity and disease treatment this makes it a potential probiotic. Finally the LAB isolated from curd demonstrated probiotic attributes with good antimicrobial activities invitro therefore exhibiting potentially to use them as probiotics in food and feed formulation. The study was carried out to prove that the *Lactococcus* can produce antimicrobial substance to inhibit the growth of pathogenic bacteria. The furthermore studies needed to improve the works, in the future.

BIBLIOGRAPHY

Alonso, S., Carmen Castro, M., Berdasco, M., de la Banda, I. G., Moreno-Ventas,X., and de Rojas, A. H. (2018). Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. Probiotics Antimicrob. Proteins V(**11**), 569-579. doi:*10.1007/s12602-018-9439-2*

Ashraf R, Shah NP. Immune system stimulation by probiotic microorganisms.Crit Rev Food SciNutr. 2014;V**(54)**7.938-56.

Bartkiene, E., Zavistanaviciute, P., Lele, V., Ruzauskas, M., Bartkevics, V.,Bernatoniene, J., et al. (2018). Lactobacillus plantarum LUHS135 and paracasei LUHS244 as functional starter cultures for the food fermentation industry: characterisation, mycotoxin-reducing properties, optimisation of biomass growth and sustainable encapsulation by using dairy by-products. LWT V**(93)**,649–658. doi: 10.1016/j.lwt.2018.04.017

Batista, A. L. D. Silva, R., Cappato, L. P., Ferreira, M. V. S., Nascimento, K. O.Schmiele, M., et al. (2017). Developing a synbiotic fermented milk using probiotic bacteria and organic green banana flour. J. Funct. Foods **V**(**38**), 242-250 doi: 10.1016/j.iff 2017.09.037

Bron PA, Kleerebezem M. Engineering lactic acid bacteria for increased industrial functionality. Bioeng Bugs. 2011;2:80–87. V(**10**).4161/bbug.2.2.13910.

Chavan, M.; Gat, Y.; Harmalkar, M.; Waghmare, R. Development of non-dairy fermented probiotic drinkbased on germinated and ungerminated cereals and legume. LWT-Food Sci. Technol. 2018,V(**91**), 339–344.[CrossRef]

Chang CK, Wang SC, Chiu CK, Chen Sy, Chen ZT, et al. (2015) Effect of lactic acid bacteria isolated from fermented mustard on immunopotentiating activity. Asian Pac J Trop BiomedV**(5)**: 281-286.

Coeuret V, Dubernet S, Bernardeau M, Gueguen M, Vernoux JP. Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Le Lait, INRA Editions 2003; V(**83**): 269–306.

Coste M., Rochet V., Leonil J., Molle D., BouhallabS.,*Tome D. (1992).**Identification of C-terminal peptides of bovine b-casein that enhance proliferationof rat lymphocytes.Immunol.Lett.,V(33), pp. 41-46.**

Dowarah R, Verma AK, Agarwal N, Singh P, Singh R.Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. plos one. 2018;13:e0192978. doi:V**(10)**.1371/journal.pone.0192978.

FAO/WHO Working Group Report.(2002).*Guidelines for the evaluation of probiotics* in food. London, Ontario, Canada.V**(1)**

Fuller R. Probiotic in man and animals. A*Review J ApplBacteriol1989V**(90)**3452-3*.

Ghosh, K.; Ray, M.; Adak, A.; Dey, P.; Halder, S.K.; Das, A.; Jana, A.; Parua, S.; Das Mohapatra, P.K.; Pati, B.R.; et al. Microbial, saccharifying and antioxidant properties of an Indian rice based fermented beverage. Food Chem. 2015, V**(168)**, 196-202. [CrossRef]

Hannu K. (2009). *Milk-derived bioactive peptides: From science to applications.*

*J. Funct. Foods, 1, pp. 177-187. Nielsen M. S., Martinussen T., Flambard B., Sorensen K.I., Otte J. (2009). Peptide profiles and angiotensin-l-converting enzyme inhibitoryactivity of fermented milk products: Effect of bacterial strain, fermentation pH, and storage time. Int. Dairy J., V**(19)**, pp. 155-165.*

Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastro. Hepat. 2014, V**(11)**, 506-514. [Crossk.f]

Kechagia, M.; Basoulis, D.; Konstantopoulou, S.; Dimitriadi, D.; Gyftopoulou, K.; Skarmoutsou, N.; Fakiri, E.M. Health benefits of probiotics: A review. ISRN Nutr. 2013, 2013, doiV**(10)**.5402/2013/481651.

Kim, Y., Beuchat, L.R.; Ryu, J.H. Reduction of Bacillus cereus spores in sikhye, a traditional Korean rice beverage, by modified tyndallization processes with and without carbon dioxide injection. Lett. Appl. Microbiol. 2012, V**(55)**, 218-223. [Cross Ref]

Klein G, Pack A, Bonaparte C, Reuter G. Taxonomy and physiology of probiotic lactic acid bacteria. Int J Food Microbiol 1998: V**(41)**: 103-25.

Leroy F, De Vuyst L. Functional lactic acid bacteria starter cultures for the food fermentation industry. Trends in Food Science and Technology 2004; V**(15)** 6778.

Louwehand AC, Röytiö H. Probiotic fermented foods and health promotion: Elsevier, 2014V**(1)**

Mahalot, A andMandal, S. (2018). Assessment of lactic acid bacteria from cow milk and goat milk samples for probiotic potentiality by in vitro methods. Acta Scientific Pharmaceutical Sciences, V**(2)**: 56-58.

Maresca D, Zotta T, Mauriello G. Adaptation to aerobic environment of Lactobacillus johnsonii/gasseristrains. Front Microbiol. 2018;V**(1)**157. doi:10.3389/(micb.2018.00157 5.

Ogunremi, O.R.; Agrawal, R.; Sanni, A.I. Development of cereal-based functional food using cereal-mixsubstrate fermented with probiotic strain-Pichiakudriavzevii OG32. Food Sci. Nutr. 2015, V**(3)**, 486-494. [CrossRef]

Olkowski AA, Wojnarowicz C, Nain S, Ling B. Alcorn JM, Laarveld B (2008).A study on pathogenesis of sudden death syndrome in broiler chickens. Res. Vet. Sci.V**(85)**:131-140.

Parvez S, Malik KA, Ah Kang S, Kim HY.Probiotics and their fermented food products are beneficial for health. J ApplMicrobiol 2006;V**(1)** 100: 1171-85.

Pena-Ramos E. A., and Xiong Y. L. (2001).*Antioxidative activity of whey protein hydrolysates in a liposomal system. J. Dairy Sci., V**(84)**, pp. 2577-2583.*

Peyer, L.C.; Zannini, E.; Arendt, E.K. Lactic acid bacteria as sensory biomodulators for fermented cereal-basedbeverages. Trends Food Sci. Technol. 2016, V**(54)**, 17-25. [Cross Ref]

Probiotics Market—Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast, 2012–2018. Available online: http://www.transparencymarketresearch.com/ probioticsmarket.htm/2013 (accessed on 24 October 2014)V**(1)**.

Renzo, T.D.; Reale, A.; Boscaino, F.; Messia, M.C. Flavoring production in Kamut®, quinoa and wheat doughs fermented by Lactobacillus paracasei, Lactobacillus plantarum, and Lactobacillus brevis: A SPME-GC/MS Study. Front. Microbiol. 2018, V**(9)**,429. [Cross Ref]

Shehata, M.G., Sohaimy S.A.E., Malak, A and Youssef, E.M.M. 2016).

Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydro lase activity. Annals of Agricultural Science,V**(61)** 65-75.

Spyropoulos, B.G.; Misiakos, E.P.; Fotiadis, C.; Stoidis, C.N. Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. Dig. Dis. Sci. 2011, V**(56)**, 285–294. [CrossRef]

***Son S., Lewis B. A. (2002)**. Free radical scavenging andantioxidative activity of caffeic acid amide and ester analogues: Structure-activity relationship. J. Agric. Food Chem.,V**(50)**, pp. 468-472.*

Tomovska J., Stojanoski Z., Tomovska N. (2010). ACE in *hibitory peptides in fermented milk products and arterial blood pressure. Ultra Scientist of Physical Sciences,V**(22)**,* pp. 661-674**Tomovska J., Presilski S., Gjorgievski N., Tomovska N., *Qureshi S. M.,andBozinovska P. N. (2013)**. Development of a spectrophotometric method for monitoring angiotensin-converting enzyme in dairy products. Pak. Vet. J. V**(33)**,* pp. 14-18.