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An Experimental Review on "Detection of Quality of Milk, Isolation of Microbial Protein and Detection of Its Antimicrobial Properties."

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Abstract

Dairy activities have traditionally been integral to India's rural economy. The country is the world's largest producer of dairy products and also their largest consumer. Almost its entire produce is consumed in the domestic market and the country is neither an importer nor an exporter, except in a marginal sense. Despite being the world's largest producer, the dairy sector is by and large in the primitive stage of development and modernization. Though India may boast of a 200 million cattle population, the average output of an Indian cow is only one seventh of its American counterpart. Indian breeds of cows are considered inferior in terms of productivity. Moreover, the sector is plagued with various other impediments like shortage of fodder, its poor quality, dismal transportation facilities and a poorly developed cold chain infrastructure. As a result, the supply side lacks in elasticity that is expected of it.

On the demand side, the situation is buoyant. With the sustained growth of the Indian economy and a consequent rise in the purchasing power during the last two decades, more and more people today are able to afford milk and various other dairy products. This trend is expected to continue with the sector experiencing a robust growth in demand in the short and medium run. If the impediments in the way of growth and development are left unaddressed, India is likely to face a serious supply – demand mismatch and it may gradually turn into a substantial importer of milk and milk products. Fortunately, the government and other stakeholders seem to be alive to the situation and efforts to increase milk production have been intensified. Transformations in the sector are being induced by factors like newfound interest on the part of the organized sector, new markets, easy credit facilities, dairy friendly policies by the government, etc. Dairy farming is now evolving from just an agrarian way of life to a professionally managed industry – the Indian dairy industry. With these positive signals, there is hope that the sector may eventually march towards another white revolution (Baumal R, Musclow 1990).

Keywords : Dairy activities, White revolution, Domestic market, Milk and milk products, Dairy farming.

Production of Milk In Industries

Dairy activities have traditionally been integral to India's rural economy. The country is the world's largest producer of dairy products and also their largest consumer. Almost its entire produce is consumed in the domestic market and the country is neither an importer nor an exporter, except in a marginal sense. Despite being the world's largest producer, the dairy sector is by and large in the primitive stage of development and modernization. Though India may boast of a 200 million cattle population, the average output of an Indian cow is only one seventh of its American counterpart. Indian breeds of cows are considered inferior in terms of productivity. Moreover, the sector is plagued with various other impediments like shortage of fodder, its poor quality, dismal transportation facilities and a poorly developed cold chain infrastructure. As a result, the supply side lacks in elasticity that is expected of it.

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History

The development of Indian dairy industry took its shape after the white revolution. The white revolution has increased the milk production from 17 million tons (1950-1951) to 110 million tons (2008-2009).

Dr. Verghese Kurien engineered the white revolution in India. He was the chairman of the Gujarat Co-operative Milk Marketing Federation Ltd. (GCMMF) and his name was synonymous with the Amul brand. Operation Flood (white revolution), was done in 3 phases.

Operation Flood

Operation Flood has helped farmers, direct their own development, placing control of the resources they create in their own hands. A „National Milk Grid", bridges milk producers throughout India with consumers in over 700 towns and cities, reducing seasonal and regional price variations while ensuring that the producer gets a major share of the price consumers pay (Farkas-Himsley H.,1991).

Programme Implementation

Operation Flood was implemented in three phases.

Phase I

Phase I (1970 –1980) was financed by the sale of skimmed milk powder and butter oil donated by the European Union (then the European Economic Community) through the World Food Programme. NDDB planned the programme and negotiated the details of EEC assistance.

During its first phase, Operation Flood linked 18 of India's premier milk sheds with consumers in India's major metropolitan cities: Delhi, Mumbai, Kolkata and Chennai; thus establishing mother dairies in four metros. Operation flood, also referred to as "White Revolution" was a gigantic project propounded by Government of India for developing dairy industry in the country. The Operation Flood – 1 originally meant to be completed in 1975, actually the period of about nine years from 1970-79, at a total cost of Rs.116 crore. As start of operation Flood-1 in 1970 certain set of aims were kept in view for the implementation of the programmers. Improvement by milk marketing was made by organizing dairy sector in the metropolitan cities including Mumbai, Calcutta, Chennai, and Delhi. The objectives of commanding share of milk market and speed up development of dairy animals respectively hinter lands of rural areas with a view to increase both production and procurement.

Phase II

Operation Flood Phase II (1981 – 1985) increased the milk sheds from 18 to 136;290 urban markets expanded the outlets for milk. By the end of 1985, a self-sustaining system of 43,000 village cooperatives with 42.5 lakh of milk producers were covered. Domestic milk powder production increased from 22,000 tons in the pre-project year to1, 40,000 tons by 1989, and the increase coming from dairies set up under Operation Flood. In this way EEC gifts and World Bank loan helped promote self-reliance. Direct marketing of milk by producer's co-operatives has increased by several million liters a day.

Phase III

Phase III (1985 – 1996) enabled dairy cooperatives to expand and strengthen the infrastructure required to procure and market increasing volumes of milk. Veterinary first-aid health care services, feed and artificial insemination services for cooperative members were extended, along with intensified member education. Operation Flood's Phase III consolidated India's dairy cooperative movement, adding 30,000 new dairy cooperatives to the 42,000 existing societies organized during Phase II. Milk sheds peaked to 173 in 1988-89 with the numbers of women members and Women's Dairy Cooperative Societies increasing significantly.

Phase III gave increased emphasis to research and development in animal health and animal nutrition. Innovations like vaccine for Theileriosis, bypassing protein feed and urea-molasses mineral blocks, all contributed to the enhanced productivity of milk animals (Hammami R *et al* 2010).

Advantages

- The industrial production of milk is a boon to Indian economy because it is growing at double the rate of consumer market and is provided to the consumer at reasonable rate.
- A boom is forecast in the market of dairy products used as raw material in pharmaceuticals and allied industries.
- The increasing away from home consumption trend opens new vistas for ready-to-serve dairy products which would ride piggyback on the fast food revolution sweeping the urban India.
- The milk producing industries provide a station for the training programmes to the students of various branches.
- The industries also provide opportunities in a field of biotechnology like Daity cattle breeding of the finest buffaloes and hybrid cows; increasing milk production with recombinant somatotropin; production of probiotics, dairy biologics, enzymes and coloring materials for food processing.
- Most of all it provide employment to lot of labour class people and thus help in removing poverty from our country.

Adulteration In Milk

Quality control test for milk are very important to adulterant free milk for consumption. Adulteration of milk reduces quality of milk and can even make it hazardous. Adulterants like soap, acid, starch, table sugar and chemicals like formalin may be added to the milk. Most of the chemicals used as a adulterants are poisonous and cause health hazards. Adulterants are usually added to increase the shelf life of milk. Some of the preservatives like acid and formalin is added to milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to milk to increase the volume content of the milk. Some of the common adulterants found in milk and their detection are :

Microorganism

Milk contains some harmful microorganism like bacteria along with some potentially beneficial microbes. Microbiologically analysis of milk is carried out to determine the degree of bacterial contamination in milk and to understand the chemical changes brought in the milk as a result of microbial action. Pasteurization is done to destroy such harmful bacteria, if pasteurization is not done properly there will be the count of large amount of bacteria in the milk. **Methylene Blue Reduction Test** is used to detect the presence of bacteria in milk. This test works on the principle that the methylene blue indicator is present in an oxidized form, but in the presence of bacteria, leads to the reduction of this indicator in a comparatively short span of time. The blue color developed on addition of the indicator to the milk will change to white color within a short period indicates the presence of bacteria in the milk and thus denotes improper pasteurization.

Table Sugar

The common sugar present in milk is lactose. The fat content of the milk is more compared to the protein content. Table sugar like sucrose is added to the milk to increase the carbohydrate content of the milk and thus the density of milk will be increased. So the milk can now be adulterated with water and it will not be detected during the lactometer test. **Ketose sugar will react with the resorcinol to give a red colored precipitate**, indicating the presence of Table sugar in milk.

Acids

Generally acids like Benzoic acid and Salicylic acid is used as a preservative in food industry. It is added to milk to preserve and thus increase the shelf life of milk. Presence of these acids can be detected by adding conc.sulphuric acid and ferric chloride, which when reacts with benzoic acid and salicylic acid to give buff colored and violet colored reaction products respectively.

Soap

Soap is added to milk to increase the foaming of milk and thus to have thick milk. Addition of such chemicals will cause health problem especially related to stomach and kidneys. Soap can be detected by adding phenolphthalein indicator to the adulterated milk. A pink color will be observed if soap is present as the alkali will be neutralized by the acidity of the milk when phenolphthalein indicator is added.

Contamination In Milk

Quality control tests for milk are very important to assure adulterant free milk for consumption. Adulteration of milk reduces the quality of milk and can even make it hazardous. Adulterants like soap, acid, starch, table sugar and chemicals like formalin may be added to the milk. Most of the chemicals used as adulterants are poisonous and cause health hazards. Adulterants are mainly added to increase the shelf life of milk. Some of the preservatives like acid and formalin are added to the milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to the milk to increase the volume content of the milk.

Milk contains relatively few bacteria when it leaves the udder of healthy cow, and generally these bacteria do not grow in milk under the usual condition of handling. However, Streptococci and Micrococcus have been recovered from aseptically drawn milk. Probably the two most significant sources of contamination are dairy utensils and milk contact surfaces, including the milk pail or milking machine, as the case may be, strainers, milk cans or pipelines and the bulk milk cooler. If dairy utensils or the milk contact surfaces are inadequately cleaned, sanitized, and dried, bacteria may develop in large number in the dilute milk like residue and enter the next milk to touch this surface. Undesirable bacteria from these sources include lactic, streptococci, coli form bacteria, psychotropic gram-negative rods, and thermodurics, those which survive pasteurization, e.g. *Micrococci, Enterococci, Bacilli* and *Brevibacteria*. Application of quaternary ammonium compounds as sanitizing agent tends to increase the percentage of gram-negative rods on utensils (psychrotrophs, coliforms), whereas hypochlorides favour gram-positive bacteria (*Micrococci, Bacilli*).

Other sources of contamination after milk leaves the farm include the tanker truck, transfer pipes, sampling utensils, and other equipments at the market milk plant, cheese factory, condesery, or other processing plant. Again the most significant sources of contamination are milk-contact surfaces. Pipelines, vats, tanks, pulps, valves, seperators, clarifiers, homogenizers, coolers, strainers, stirrers and fillers may serve as possible sources of bacteria. The amount of level of contamination from each of these sources depends on cleaning and sanitizing methods.

Preservation

The various method of preservation of milk causing certain undesirable changes in milk. Following are some of the common methods use for the preservation of milk:

- Asepsis
- Pasteurization
- Freezing
- Drying
- Use of Preservatives

Asepsis

Keeping quality is usually improved when smaller numbers of microbes are present, especially those which grow readily in milk. Although the type of microorganism present is extremely those which grow readily in milk. Although the type of microorganism present is extremely important, in general, the lower the initial total microbial load, the better the keeping quality of milk. For example, a low microbial load, particularly the number of spores present, is an important consideration in the milk to be processed by ultrahigh-temperature or commercial sterilization processes

Pasteurization

The process of pasteurization involves heating of milk in large tanks to 60C for 20 minutes. The use of plate heat exchangers and continuous operation involves the high temperature short time (HTST) pasteurization process at the temperature of atleast 72 C for atleast 15 seconds. Heat-treatment processes in excess of pasteurization for milk and milk products have been designated as very high temperature (VHT) systems and ultrahigh-temperature (UHT) systems. The most popular UHT systems are direct-heating methods, including a steam injection into milk pricess and a milk injected into steam process referred to as steam injection technique and steam infusion technique, respectively.

Freezing

At very low temperature, the growth of microorganism is impossible. The microbial content of ingredients- milk, cream, sugar, eggs, stabilizers, and flavouring and coloring materials along with contamination picked up during processing will determine the numbers and kinds of microorganism in the mix and the microbial content after pasteurization of the mix and freezing.

Drying

Various milk products are made by removing different percentages of water from whole or skim milk. Only in the manufacture of dry products is enough moisture removed to prevent the growth of microorganism. The reduction in moisture and consequent increase in the concentration of dissolved substances in liquid condensed milk products inhibits the growth of some kinds of bacteria.

Useful bacteria found in milk (lacto bacillales)

Taxonomy

KINGDOM: BACTERIA

PHYLUM : FIRMICUTES

CLASS : BACILLI

ORDER : LACTOBACILLALES

FAMILY : LACTOBACILLACEAE

GENUS : *LACTOBACILLUS*

Morphology

Lactobacillales or lactic acid bacteria (LAB) are a clade of Gram-positive, low-GC, acid-tolerant, generally nonsporulating, nonrespiring, either rod- or cocci-shaped bacteria that share common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end product of carbohydrate fermentation. This trait has, throughout history, linked LAB with food fermentations, as acidification inhibits the growth of spoilage agents. (J.P. Euzéby) Proteinaceous bacteriocins are produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic microorganisms. Furthermore, lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item. The industrial importance of the LAB is further evinced by their generally recognized as safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to the healthy microflora of human mucosal surfaces. The genera that comprise the LAB are at its core *Lactobacillus*, Leuconostoc, Pediococcus, Lactococcus and *Streptococcus*, as well as the more peripheral Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Tetragenococcus, Vagococcus, and Weissella; these belong to the order Lactobacillales. (Mc Grath S and van Sinderen D)

Figure: Colonies of *Lactobacillus*

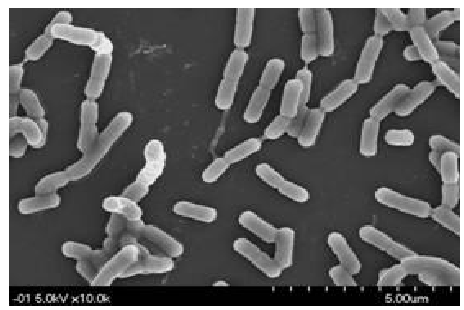

Figure: Morphology of *Lactobacillus*

The lactic acid bacteria (LAB) are either rod-shaped (bacillus), or spherical (coccus), and are characterized by an increased tolerance to acidity (low pH range). This aspect helps LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production (e.g., lactic acid). Laboratory media used for LAB typically include a carbohydrate source, as most species are incapable of respiration. LAB are catalase negative. They consist of the organelles of a simple bacterial structure. LAB are amongst the most important groups of microorganisms used in the food industry (Sonomoto, K; Yokota, 2011).

Uses Of Lactobacillales

Various different *Lactobacillus* species have roles in the production of various human foods and beverages, including:

In Production Of Various Human Foods And Beverages

Figure: Various Milk Products

- yoghurt
- sourdough breads
- cheese
- Sauerkraut
- pickles
- wine
- cider
- Kimchi

In Medical Field

- Relieves constipation
- Reduces flatulence
- Relieves urinary tract infection
- Reduces/prevents heartburn
- Protects against indigestion
- Prevents stomach ulcers
- Kills deadly strains of *E coli* and *Salmonella*
- Protects against cancer
- Prevents/protects against cardiovascular disease

- Helps reduce symptoms of irritable bowel syndrome
- Relieves bloating
- Prevents thrush
- Reduces risk and symptoms of ulcerative colitis
- Improves hypersensitivity reactions among infants with food allergies
- Prevents diarrhea
- Reduces abdominal/gastrointestinal cramping
- Combats vaginal yeast infections
- Reduces bad breath
- Encourages production of immune system cells to fight viral infections
- Protects against colon cancer
- Prevents development of diverticulitis
- Protects against and relieves symptoms of Crohn's disease
- *L. acidophilus* appears to have a role in healthy women in maintaining the vaginal pH, but studies have not shown the effectiveness of orally-ingested supplements or tablets on altering the vaginal microflora.
- It is also claimed that *L. acidophilus* can help boost an individuals immune system, however studies on this seem to produce mixed results and are inconclusive. There does, however, seem to be some validity (supported by research) suggesting that *L. acidophilus* can have an effect on reducing the presence of undesirable micro-organisms within the intestine, as a result of its lowering of the intestinal pH. This effect is facilitated because of the bacterium's ability to survive very low pHs, such as are present in the stomach, and so be able to pass to lower regions of the human gastrointestinal tract (Welman *et al* 2009).

Lactobacillus Protein (Bacteriocin)

A great number of Gram (+) and Gram negative (-) bacteria produce during their growth, substances of protein structure (either proteins or polypeptides) possessing antimicrobial activities, called bacteriocins. Although bacteriocins could be categorized as antibiotics, they are not. The major difference between bacteriocins and antibiotics is that bacteriocins restrict their activity to strains of species related to the producing species and particularly to strains of the same species, antibiotics on the other hand have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains. In addition, bacteriocins are ribosomally synthesized and produced during the primary phase of growth, though antibiotics are usually secondary metabolites. Among the Gram (+) bacteria, lactic acid bacteria (LAB) especially, LACTOBACILLI have gained particular attention nowadays, due to the production of bacteriocins. These substances can be applied in the food industry as natural preservatives. The use of LAB and of their metabolic products is generally considered as safe (GRAS, Grade One). The application of the produced antimicrobial compounds as a natural barrier against pathogens and food spoilage caused by bacterial agents has been proven to be efficient. Nisin is the only bacteriocin that has been officially employed in the food industry and its use has been approved worldwide. Bacteriocins (IJFM 2006) can be applied on a purified or on a crude form or through the use of a product previously fermented with a bacteriocin producing strain as an ingredient in food processing or incorporated through a bacteriocin producing strain (starter culture).

Classification of Bactriocin

Bacteriocins are categorized in several ways, including producing strain, common resistance mechanisms, and mechanism of killing. There are several large categories of bacteriocin which are only phenomenologically related. These include the bacteriocins from gram-positive bacteria, the colicins,[] the microcins, and the bacteriocins from Archaea. The bacteriocins from *E. coli* are called colicins (formerly called 'colicines,' meaning 'coli killers'). They are the longest studied bacteriocins. They are a diverse group of bacteriocins and do not include all the bacteriocins produced by *E. coli*. For example, the bacteriocins produced by *Staphylococcus warneri* are called as warnerin or warnericin. In fact, one of the oldest known so-called colicins was called colicin V and is now known as microcin V. It is much smaller and produced and secreted in a different manner than the classic colicins. This naming system is problematic for a number of reasons. First, naming bacteriocins by what they putatively kill would be more accurate if their killing spectrum were contiguous with genus or species designations. The bacteriocins frequently possess spectra that exceed the bounds of their named taxa and almost never kill the majority of the taxa for which they are named. Further, the original naming is generally derived not from the sensitive strain the bacteriocin kills, but instead the organism that produces the bacteriocin (IDJ 2006). This makes the use of this naming system a problematic basis for theory; thus the alternative classification systems (Dimov S., I.P., Harizanova N 2005).

Uses of Bacteriocin

Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body. Bacteriocins have also been suggested as a cancer treatment. They have shown distinct promise as a diagnostic agent for some cancers, but their status as a form of therapy remains experimental and outside the mainsteam of cancer research. This is partly due to questions about their mechanism of action and the presumption that anti-bacterial (IJFM 2001) agents have no obvious connection to killing mammalian tumor cells. Some of these questions have been addressed, at least in part. Bacteriocin were tested as AIDS drugs around 1990, but did not progress beyond in-vitro tests on cell lines. Bacteriocins (IDJ 2006) have been proposed as a replacement for antibiotics to which pathogenic bacteria have become resistant. Potentially, the bacteriocins could be produced by bacteria intentionally introduced into the patient to combat infection (Micron Journal 1996).

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