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Research article

ISOLATION OF *LACTIC ACID BACTERIA* UNDER LOW TEMPERATURE FOR THE PREPARATION OF YOGURT

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ABSTRACT:

Objective: An investigation of isolation of Lactic acid bacteria was carried out under low temperature for the preparation of Yogurt by using various food supplements like carrot, ground-nut and tomato juices. **Methods**: Various samples of Cow milk, Skimmed milk were processed along with nutrients like Carrot, ground nut and tomato juices with Tryptone glucose yeast extract agar (TGYA) at different temperatures like 5^0C, 15^0C and 22^0C for the isolation of *Lactic acid bacteria* for the preparation of yogurt. The characteristic isolates were identified by using various biochemical tests and direct microscopy. **Results**: *Lactic acid bacteria* (LAB) dominated the microbial population of Yogurt, and were identified according to their morphological and physiological characteristics. Among these *lactobacilli* were frequently occurring organisms. The most abundant species were *Lactobacillus delbrueckii* subspecies *Bulgaricus* and *Streptococcus thermophilus.* The Lactic *Streptococci* was subjected to bio-chemical tests to identify the species. Based on the biochemical reactions the species was identified as *Lactococcus Lactis*, sub species *di-acetylactis*. Isolated culture of lactic *Streptococci* was found to grow at low temperature. When this was used as an inoculum to prepare yogurt at 5^0C, 15^0C and 22^0C curdling took place in 3days time. In order to reduce the setting time, nutrients in the form of carrot, ground-nut and tomato juices were added. The yogurt was found to set at 5^0C in 30hrs which is considered useful. Acidity of yogurt was found to be 0.53%- 0.55%. The yogurt was found to contain di-acetyl and quality of yogurt was good.

Key Words: Yogurt, LAB, TGYA, *Streptococcus thermophilus*

INTRODUCTION

Lactic acid bacteria (LAB) are one of the microorganisms that dominate fermented food (Guasch-Jané 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 (Temmerman et al. 2002) and stimulation of the immune system (Kalliomäki et al. 2001) render this group of microorganism's essential importance in dairy industry.

Yogurt 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. thermophilus* and *L. delbrueckii* ssp. *Bulgaricus* (Masud, T 1991, Raquib M., et al. 2003). Bacterial species currently used in the dairy industry belong to the genera *Lactobacillus* and *Streptococcus. Lactic acid bacteria* (LAB) form a phylogenetically diverse group and are defined as Gram-positive, non-sporing, catalase-negative, fastidious, acid tolerant and strictly fermentative bacteria that 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. However, new approaches for identification of LAB by physicochemical methods such as SDS-PAGE protein patterns (Kersters, 1980) have found wide applications. Since yogurt is prepared from undefined heterogeneous mixture of LAB strains, the determination of plasmid profile offers new possibilities for differentiating the isolates on the strain level (Sewaki et al., 2001). The identification of LAB was performed according to their morphological, cultural and biochemical characteristics as described by (Collins, 1980). The quality of yogurt available in the market or prepared in the household is however subjected to considerable micro-organisms which may cause various defects like gassiness, off-flavours and proteolysis in the product. Other factors which influence the quality of yogurt are purity and activity of the culture, 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 Yogurt from various sources such as utensils, hands of persons and atmosphere of the kitchen. Under laboratory conditions all these factors could be controlled and selected culture of lactic acid bacteria of known characteristics could be used to obtain yogurt of consistently uniform quality having the desired acidity and flavour. As no such study has been carried out on lactic acid bacteria in this region, the aim of this study is to isolate indigenous strains of lactic acid bacteria for the preparation of yogurt under low temperature. The optimum temperature for preparation of yogurt is around 20^0 -30^0C. Under low temperature conditions it is difficult to make fermented yogurt and therefore there is need to isolate suitable lactic cultures capable of setting the yogurt at temperatures like 5^0C, 15^0C and 22^0C. There are several advantages in developing a method 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 temperature. A method for the preparation of yogurt at low temperature has been standardized using a lactic culture isolate. The yogurt sets at 5^0C, 15^0C and 22^0C with mild acidity. The yogurt can be stored for a long time without significant increase in the acidity. The yogurt can be prepared at house-hold 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 yogurt with characteristic flavour due to di-acetyl production.

MATERIALS AND METHODS

The isolation of the desired organism was performed on solid selective media. The streak plate method was used to isolate the lactic acid bacteria. For this purpose a loopful of each sample was streaked on MRS agar and M17 agar (Oxoid, UK) plates with 10% lactose solution and the plates were incubated at 37°C for 24hrs. After incubation, the culture was observed for growth, single and isolated colonies were picked and sub cultured on MRS agar and M17 agar media and incubated at 37°C for 24hrs to obtain pure culture of the isolates. Simultaneously the smears were prepared and stained with Gram's stain as described by (Kersters, 1980) and examined under microscope for the staining characteristics and morphology of the isolates. The identification of LAB was performed according to their morphological, cultural and biochemical characteristics as described by Collins and Lyne. Then the isolated organisms were used with various sources of milk samples like Cow milk, Skimmed milk, Carrot, ground nut, tomato juices along with Tryptone glucose yeast extract agar (TGYA) at different temperatures like 5^0C, 15^0C and 22^0C for the for the preparation of yogurt.

RESULTS

The main species which were isolated for the preparation of yogurt at low temperature include *Streptococcus thermophilus, Lactococcus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus, Lactobacillus lactis, it* was further observed that *S. thermophilus* and *L. lactis along with L.bulgaricus* constituted the dominant stains of yogurt. The Lactic *Streptococci* was subjected to bio-chemical tests to identify the species. Based on the biochemical reactions the species was identified as *Lactococcus Lactis*, sub species *di-acetylactis*. Isolated culture of lactic *Streptococci* was found grow at low temperature. When this was used as an inoculum to prepare yogurt at 5^0C, curdling took place in 3days time. In order to reduce the setting time, nutrients in the form of carrot juice was added. The yogurt was found to set at 5^0C in 30hrs which is considered useful. Acidity of yogurt was found to be 0.53%- 0.55%. The yogurt was found to contain di-acetyl and quality of yogurt was good. At 15^0C when carrot juice was added the setting time was found to be 24hrs, with an acidity ranging from 0.57%-0.58% with a pH range of 5.50-5.55, di-acetyl was present and the quality of yogurt was good. At 22^0C when carrot juice was added, the setting time was found to be 24hrs with an acidity of 0.41%-0.43% which was very mild with a pH value varying 5.49-5-61. The di-acetyl was present and the quality of yogurt was good. At 5^0C when groundnut juice was added setting time was found to be 32hrs, with an acidity of 0.53%-0.56% with a pH range of 7.1-7.8, the yogurt was found to be positive for di-acetyl and the quality of yogurt was good. At 15^0C when groundnut juice was added the setting time of yogurt was found to be 24hrs with an acidity of 0.55%-0.58% with a pH range between 5.6-5.65. Yogurt was found to be positive for the di-acetyl production and quality was acceptable. When groundnut juice was added at 22^0C the yogurt was found to be set within 24hrs of time with mild acidity 0.59%-0.61%, the pH was 5.1-5.9. Yogurt showed positive test for di-acetyl production and the quality was good. When tomato juice was added at 5^0C the setting time of yogurt was 30hrs, with an acidity ranging 4.28%-6.21%.The pH range was between 6.2-6.13, the yogurt was positive for di-acetyl production and the quality of yogurt was good. At 15^0C when tomato juice was added the setting time was found to be 24hrs with an acidity of 0.55%-0.56%. The pH range was 5.6-5.7. The di-acetyl was present and the quality was acceptable. When tomato juice was added at 22^0C the setting time was found to be 24hrs with an acidity ranging from 0.58%-0.61% while pH ranges from 5.1-5.7. The yogurt was found to be positive for di-acetyl production and the quality of yogurt was acceptable.

Table 1: Use of *Lactococcus Lactis* for the preparation of yogurt at 5^0C, 15^0C and 22^0C by using carrot juice

Trail no.	Growth media	Acidity	pH	Incubation temperature	Setting time	Di-acetyl test
1.	Milk+peptone+carrot	4.38	7.1	5^0C	30hrs	+ve
2.	Milk+peptone+carrot	0.58%	5.55	15^0C	24hrs	+ve
3.	Milk+peptone+carrot	4.5	5.56	22^0C	24hrs	+ve

Table 2: Use of *Lactobacillus bulgaricus* for the preparation of yogurt at 5^0C, 15^0C and 22^0C by using ground nut juice

Trail no.	Growth media	Acidity	pH	Incubation temperature	Setting time	Di-acetyl test
1.	Milk+peptone+groundnut	4.38	7.8	5^0C	32hrs	+ve
2.	Milk+peptone+groundnut	0.55%	5.65	15^0C	24hrs	+ve
3.	Milk+peptone+groundnut	0.6	5.90	22^0C	24hrs	+ve

Table 3: Use of *Lactobacillus Lactis* for the preparation of yogurt at 5^0C, 15^0C and 22^0C by using Tomato juice

Trail no.	Growth media	Acidity	pH	Incubation temperature	Setting time	Di-acetyl test
1.	Milk+peptone+tomato	4.28	6.7	5^0C	30hrs	+ve
2.	Milk+peptone+tomato	0.55%	5.51	15^0C	24hrs	+ve
3.	Milk+peptone+tomato	0.58%	5.14	22^0C	24hrs	+ve

DISCUSSION

The temperature of the country varies from region to region. There are several hill stations where the atmospheric temperature is below 15^0C. Under these conditions the yogurt will not set or form due to low temperature. The optimum temperature for making yogurt is 26^0C-35^0C. In order to develop lactic culture capable of growing at temperature below 15^0C, attempts were made in this investigation to isolate one such culture which is capable of setting yogurt at 5^0C and 15^0C. The yogurt that was prepared using these lactic culture isolates was subjected to sensory score of 6.5 out of 7 by Hedonic rating. This yogurt capable of setting at 5^0C and 15^0C is considered advantageous in successfully practicing these preparations in places where the atmospheric temperature is low. Further such yogurt is likely to possess long viability and shelf-life, the rate of increase in the acid development is also likely to be at a slow phase. There is no chance of yeast growing in these types of yogurts due to low temperature of incubation. The yogurt prepared by such methods in cold regions possesses various therapeutic values as reported earlier by (Sewaki et al., 200l). Quality of such yogurt will be rich in the flavour, possessing mild acidity to satisfy the long felt demand of the people living in cold regions. Yogurt is mainly produced in the Middle East and other countries around the eastern part of the Mediterranean and the southern part of East Asia particularly India, Pakistan, Bangladesh and Bhutan. It is said to have originated thousands of years ago in Eastern Europe and western Asia and is still consumed in large quantities (Holland 1989). Yogurt is a well known fermented dariy product since ancient times. The preparation of yogurt involves the use of lactic cultures known to grow at ambient temperature. In cold regions and seasons the yogurt formation is hampered unless warm temperature is provided. Two types of yogurt are available in the market sweet yogurt and sour yogurt and both are prepared by a traditional method by using previous yogurt (starter). Traditional method involves invariably production on a small scale either in the consumer's household or sweetmeat makers shop. In the household milk is heated to its boiling temperature until volume is reduced up to 15%-20% and 8-10% sugar is added (sweetened yogurt) cooled down to body temperature inoculated 2-3% starter and poured in to earthenware and kept for yogurt formation overnight by wrapping in a woolen cloth or straw bag to keep warmth. Various means and methods are adopted in its preparation, so there can be seen a lot of variation among the quality of products. Through these studies we came to know that our method of Yogurt preparation is easy and time saving, also the product can be stored for a longer time without any contamination. Yogurt is prepared mainly from cow's milk because of its availability and a small amount is prepared from buffalos milk in Bangladesh compared to a significant amount prepared from the same in India (Aneja, 1990). It has been estimated that approximately 4% of the total milk produced in the Bangladesh is utilized for yogurt preparation (Mustafa, 2002). Yogurt is a well known fermented product that is consumed in large quantities throughout the world as a part of daily diet or a beverage (Sukumar De 2000). Yogurt produced at low temperature by using Lactic Acid Bacteria has been reported to exert a possible therapeutic effect by controlling bacterial growth and to cure various intestinal disorders such as constipation, diarrhea, and dysentery because of its antibiotic activity (Sewaki et al., 200l; Collins, 1980). It has been claimed that such type of yogurt can treat various heart ailments by lowering blood cholesterol level or certain cancers (Masud, 1991; Holland, 1989).

As in 1901, Metchnikoff recognized the therapeutic virtues of sour milk caused by the *lactobacilli*. He found that the longlivety of Bulgarians was in part due to their ingesting large quantities of *Lactobacillus bulgaricus* milk, prepared with the help of *lactobacillus bulgaricus*. It is also evident from the result that the lactic acid bacteria dominated the microbial flora of yogurt. MRS and M17 media are the best suitable media for the isolation of lactic acid bacteria. The presence of *L. acidophilus* in yogurt samples is beneficial and could be used in combination with *L. delbruekii* subsp. *Bulgaricus* in the preparation of yoghurt as probiotic culture with improved organoleptic characteristics and enhanced therapeutic benefits. According to the research conducted, eating yogurt regularly can improve and strengthen ones deficiency of phosphorus as it is good for people suffering from or at risk of osteoporosis. The research also suggests that sphingolipids, conjugated linolenic acid and butyric acid found in yogurt can cure cancer. The intestine friendly bacterial cultures in yogurt can keep colon healthy and reduces the risk of colon cancer. Yogurt is a rich source of calcium, a mineral that contribute to colon health and decrease the risk of cancer (Akiyoshi Hosono 2002). Furthermore, *Lactobacillus acidophilus* is a natural inhabitant of mammalian gastrointestinal systems. This species is of considerable industrial and medical interest, because *L. acidophilus* is believed to play an important role in human health and nutrition by its influence on the intestinal flora (Roy, 2001). A method for the preparation of yogurt at low temperature has been standardized using the lactic culture isolates such as *Lactococcus Lactis*, *Lactobacillus bulgaricus and Lactobacillus Lactis.* The yogurt sets at 5^0C, 15^0C and 22^0C with mild acidity and di-acetyl production while supplying the supplements like carrot, groundnut and tomato. The major advantage in producing yogurt at low temperature is that contaminating types like yeasts and moulds cannot grow. The yogurt can be stored for a long time without significant increase in the acidity. The yogurt can be prepared at house-hold conditions using this culture in cold regions during winter season. This method of Yogurt preparation is easy and time saving.

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