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Original Article

Study of Natural Starter Culture of Lactic Acid Bacteria in fermented Tunisian milk “Rayeb”

Olfa Samet Bali*, Imène Felfoul, Rouaa Lajnef, Hamadi Attia and Mohamed Ali Ayadi

Alimentary Analysis Unit, National Engineering School of Sfax, BPW 3038, Sfax, Tunisia.

*Corresponding author. E-mail: olfabali@yahoo.fr

Tel: + 216 97 556 136. Fax: + 216 74 675 761.

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Abstract

Fifty samples of traditional fermented milk “Rayeb” were randomly collected from villages near Sfax governorate, Tunisia and subjected to determination of pH, sensory evaluation and isolation and identification of lactic acid bacteria. The obtained results revealed that pH ranged from 3.7 to 4.4 with a mean of 3.93 ± 0.04 , most of the examined samples (46%) were judged as very good. The isolated lactic acid bacteria were lactobacillus, lactococcus, leuconostoc and streptococcus as 14.70, 40.20, 33.34 and 11.76%, respectively, the majority of identified isolates were *Lact. Lactis subsp. Lactis* (36%), *Leuco. mesen cremoris* (28%) followed by *St. salivaris var thermophilus* (12%), both *L. delbrukii var bulgaricus* and *L. acidophilus* represented as (9%) while the lowest percent (6%) was *L. delbrukii var delbrukii*. There are several factors which influence the quality of fermented milks, these include type of milk, processing conditions, storage conditions and however, quality of starter culture is the most important factor that influences the development of fermented milks. The choice of starter culture being based on their production of lactic acid, aromatic compounds as well as their therapeutic values to some types used.

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Key words: Fermented Milks; Rayeb; Natural Starter Culture; pH; Sensory Evaluation.

1. INTRODUCTION

Fermented milks are products prepared by controlled fermentation of milk to produce acidity and flavor to desirable level. Fermented milks are the most common products from which other products are also made [1]. Starter culture organisms used in fermentation belong to a family of bacteria collectively known as the Lactic Acid Bacteria (LAB). These LABs are united by a constellation of morphological, metabolic and physiological characteristics.

There are several factors which influence the quality of fermented milks, these include type of milk, processing conditions, storage conditions and however, quality of starter culture is the most important factor that influences the development of fermented milks. Lactic acid bacteria are widely distributed in nature and occur naturally as indigenous micro-flora in raw milk and fermented milks.

The dairy industry has developed considerably. The choice being based on their production of lactic acid, aromatic compounds, bacteriocins and their resistance to phages [2].

Industrialization of the biological transformation of food stuffs has increased the economic importance of lactic acid bacteria, because they play crucial role in the sensorial and safety aspects of fermented products. It is well recognized that technological properties of fermented milks such as acidity, flavor production and viscosity in great extent are strain dependant [3]. In the fermented milk manufactured in the Tunisian villages by the dairy animals' breeders, no starter is added; fermentation is based on the spontaneous fermentation by the natural micro-flora present in raw milk. The spontaneous fermentations are difficult to control; not predictable in terms of length of fermentation and quality of products. It can produce unwanted products or products with a short shelf life and are sometimes not safe since they are liable to contamination by pathogens.

The aim of the present study was isolation and identification of a large number of lactic acid bacteria from traditionally fermented Tunisian milk “Rayeb” in order to constitute the original LAB strains and to study their technological characteristics in order to select strains of

lactic acid bacteria used as starter cultures in the manufacturing of fermented dairy products which are suitable to local conditions.

2. MATERIALS AND METHODS

2.1 Collection of Samples:

Fifty samples of fermented milk (Rayeb) were collected from villages near Sfax governorate. The samples were put in sterile bottles and kept in an insulated ice-box until they were taken to the laboratory to be immediately examined for:

Determination of pH according to Bradley *et al.* [4], Sensory evaluation according to Tamime and Robinson [5] and then isolation and identification of Lactic Acid Bacteria (LAB) according to APHA [6], Therzaghi and Sandine [7], De Man *et al.* [8], Florez *et al.* [9] and Roissart Luquet [10].

2.2 Statistical Analysis:

The data were analyzed by using Graph pad prism computer program prepared by Graph pad software Inc. USA. [11].

3. RESULTS AND DISCUSSION

Results shown in Table (1) reveal that pH ranged from 3.7 to 4.4 with a mean of 3.93 ± 0.04 . PH in lactic acid fermented foods is usually reduced to less than 4.0 and this is usually sufficient to suppress the growth of most food-borne pathogens [12, 13].

Table 1: pH values in examined fermented milk “Rayeb” samples

pH	Minimum	Maximum	Mean	±SEM
	3.7	4.4	3.93	0.04

Table 2: Descriptive results of sensory evaluation of examined milk “Rayeb” samples

Grade	Fair	Good	Very good	Excellent
Number	5	20	23	2
%	10	40	46	4

Table 2 represents the analytical grades of sensory evaluation of the examined samples which revealed that most of the samples (46%) were judged as very good, followed by 20 out of 50 samples (40%) were good and 5 samples (10%) were fair, while 2 samples only (4%) were excellent. The lactic acid bacteria isolated from examined samples of fermented milk includes in the *Lactobacillus* (14.70 %), *Lactococcus* (40.20 %), *Leuconostoc* (33.34%)

Table 3: Isolated strains from the examined fermented milk “Rayeb” samples

Organism	Lactobacilli	Lactococci	Leuconostoc	Streptococci	Total
Number	15	41	34	12	102
%	14.70	40.20	33.34	11.76	100.00

Table 4: Biochemical identification of the isolated strains from the examined fermented milk “Rayeb” samples

organism	Species	%
Streptococci	<i>St. salivaris var thermophilus</i>	12
Lactobacilli	<i>L. delbrukii var bulgaricus</i>	9
	<i>L. delbrukii var delbrukii</i>	6
	<i>L. acidophilus</i>	9
Lactococci	<i>Lact. lactis subsp. Lactis</i>	36
Leuconostoc	<i>Leuco. mesen cremoris</i>	28

and *Streptococcus* (11.76%) genera (Table 3). These results showed the heterogeneousness of fermented milk “Rayeb” at different location or climatic zones. These findings agreed with the observations of Ayad *et al.* [14]. Nearly similar results were recorded by Beukes [15]. Results in Table (4) revealed that the isolated organisms were identified as Streptococci: *St. salivaris var thermophilus* (12%), Lactobacilli: *L. delbrukii var bulgaricus* (9%), *L. delbrukii var delbrukii* (6%) and *L. acidophilus* (9%) Lactococci: *Lact. lactis subsp. lactis* (36%) and Leuconostoc: *Leuco. mesen cremoris* (28%). Nearly similar finding were recorded by Beukes [15]. Probiotics are defined as living organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition [16]. Probiotics have demonstrated the ability to decrease the incidence and duration of some types of diarrheal illnesses (e.g., antibiotic associated, *Clostridium difficile*, traveler’s and rotavirus). A meta-analysis by Van Niel *et al.* [17] concluded that “*Lactobacillus* is safe and effective as a treatment for children with acute infectious diarrhea”. Van Niel and co-workers clearly demonstrated the relationship between *Lactobacillus* dose and reduction of diarrhea in children. The meta-analysis considered nine studies involving various species of lactobacilli. It appears that people on antibiotic therapy can benefit from probiotic consumption. One negative side effect of antibiotics is that they kill beneficial, as well as undesirable, bacteria. Replenishing the flora with normal/beneficial bacteria during and after use seems to minimize intestinal disruption caused by antibiotic medications. Cremonini *et al.* [18] reviewed seven studies (881 total patients) covering probiotic mitigation of antibiotic-associated diarrhea. According to this analysis, probiotics (e.g., *Lactobacillus rhamnosus* GG) can be used to prevent antibiotic-related diarrhea but do not appear to diminish existing diarrhea symptoms. About 10 to 20% of the adult population suffers from irritable bowel syndrome (IBS), which may include symptoms of abdominal cramps, bloating, diarrhea and constipation. Some symptom relief from probiotic consumption has been reported in studies to date. Research indicates that IBS is an inflammatory response to the host’s own colonic bacteria. Displace some of the host’s bacteria with probiotics (e. g., *Lactobacillus plantarum* 299V) appears to help relieve IBS symptoms in some cases [19, 20].

People with true lactose intolerance do not produce sufficient quantities of lactase, the enzyme that hydrolyzes lactose into glucose and galactose. As a result, these people can experience diarrhea, bloating, abdominal pain and flatulence due to undigested lactose reaching the large intestine and being fermented by colonic microbes. It has been demonstrated that people with lactose intolerance can consume yogurt and other fermented dairy products with fewer symptoms, even though these products may contain levels of lactose similar to unfermented dairy products. In

addition to the yogurt starter culture, some strains of *Lactobacillus acidophilus* and bifidobacteria reduce symptoms of lactose mal-digestion, although to a lesser extent than yogurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*).

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