

Evaluation of hygiene Lighvan cheese aspects regarding *Lactococcus garvieae* bacteria relying on culture-based and molecular methods

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Abstract:

One of the pathogenic bacteria species in the genus of *Lactococcus* is *Lactococcus garvieae*. It causes human disease (endocarditis) and mastitis in dairy cows to appear. The presence of these bacteria in the raw milk products like Lighvan cheese was reported. Lighvan cheese is a traditional cheese which is made from raw sheep's milk. In this study, at first, 1500 colonies similar to Lactococcal genera isolated from different sources such as fresh grass, fresh milk, curd, fresh cheese and ripened cheese according to colony morphology. Then, the 625 isolates were selected by gram stain. The biochemical tests carried out to select 60 isolates of Lactococcal genus and the PCR was used to determine the 16S rRNA gene sequence region to identify species. Based on molecular identification 50 species, were belonged to the Lactococcal genera and finally two species of *Lactococcus* were *Lactococcus garvieae*. According to the results, the presence of two species of *Lactococcus garvieae* (fresh milk and curd) was demonstrated in the early stages of Lighvan cheese production, But, there was no presence of the bacteria in fresh cheese and ripened cheese.

Keywords: 16S rRNA, biochemical tests, *Lactococcus garvieae*, Lighvan cheese, pathogenic

I. INTRODUCTION

In general, the microbial diversity of raw milk cheeses, in compared with pasteurized milk cheeses is very variable. [1]. *Lactococcus garvieae* is the species of Lactococcal genera which cause human disease (endocarditis) and mastitis in dairy cows to appear. [2, 3]. Therefore, it seems that every study on the presence of this bacteria in the raw milk products like Lighvan cheese, is important.

growth conditions of *Lactococcus garvieae* (temperature and resistance to salt concentration), is very similar to *Lactococcus lactis* species [4]. Therefore, there is a possibility of error in the identification of *Lactococcus* species regarding biochemical tests. The objective of this study was to evaluate hygiene Lighvan cheese aspects according to culture-based and molecular methods (16srRNA). [5, 6, 7, 8].

II. MATERIALS AND METHODS

A. Culture-based methods

10 g of each sample with 90 ml of sterile Ringer solution was completely homogenized by Homogenizer (Stomacher 400 Seward co.) then the samples were diluted [9]. Surface culture method was carried out by specific culture media (MRS agar and M17 agar) and Plates were incubated for 24 hours at 30°C and 37°C [10]. After incubation, colonies were evaluated in terms of the morphological and physical characteristics including color, size, location (superficial or deep), overhang (flat, convex and concave) and shape (spherical, oval and wrinkled) [4].

Suspicious colonies of *Lactococcus lactis* were selected, based on Gram staining and observation under the microscope regarding morphology of the bacteria. Also, biochemical tests were carried out in the MRS broth medium regarding growth at 10 and 45 °C, and NaCl concentrations (4 and 5/6 percent) [4].

B. Molecular methods

DNA of suspected isolates were extracted by Qiagen kit and PCR reactions were carried out under standard conditions for the identification of 16srRNA region by universal primers of lactic acid bacteria [5, 10].

III. RESULTS AND DISCUSSION:

According to biochemical tests (Table 1), 60 isolates were detected among Lactococcal genus. Then to identify accurate species of bacteria, molecular method (PCR) were used to determine of 16S rRNA sequence region.

Table 1: Physiological properties of *lactococcus garvieae*

Properties	<i>Lactococcus garvieae</i>
Growth at 10°C	+
Growth at 45°C	-
Growth in 4% NaCl	+
Growth in 6.5% NaCl	-

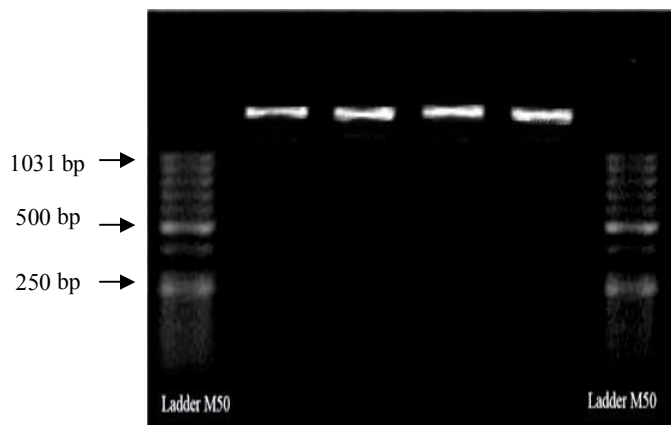


Figure 1: PCR amplicon on the agarose gel with 50bp DNA ladder

In this study, only the presence of 2 *Lactococcus garvieae* (fresh milk and curd) in the early stages of cheese production is demonstrated. But in the fresh and ripened cheese, there was no presence of the *Lactococcus garvieae*. The results indicated that Lighvan cheese has a good safety regarding these bacterial pathogen a gradual increase of acidity along with low temperature during the ripening of cheese can be important deterrent to survive *Lactococcus garvieae* [5]. On the other hand, presumably high concentration of salt in Lighvan cheese during ripening cause the number of *Lactococcus garvieae* gradually to reduce[4].

The similar studies showed that *Lactococcus garvieae* strains are more susceptible to the stressful conditions (acidity, low temperature) of ripening than *Lactococcus lactis*. [5].

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