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ANIMAL SCIENCE

Effect of Cameline Nisin isolated from *Lactococcus lactis* sub sp. lactis on *Staphylococcus aureus* sp.

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Abstract

The camel milk differs from milk of other species by the presence of a powerful protector system, linked to relatively high levels of lysozyme, lactoperoxidase, lactoferrin, component-3 of proteose peptone (PP3), organic acid, hydrogen peroxide and bacteriocins produced by lactic acid bacteria. The study aims to estimate the part of a bacteriocin (type nisin) produced by a strain of *Lactococcus lactis* sub sp lactis isolated from camel milk, in this protective system and its antibacterial activity against a strain of *Staphylococcus aureus* isolated from a bovine milk sample. Optimize conditions for a nisin production, by the producing strain isolated and purified, was carried out, by glucose addition (1%) to the M17 medium. Antagonism tests by the disk method showed inhibition zones (IZ Ø) from 6 to 8mm in diameter depending on the incubation time of the producing strain. Thus, for incubation periods of 18h, the production of camel nisin appeared to be maximum (IZ= Ø 8mm) with a minimum growth of *Lactococcus lactis* sub sp lactis (pH 6, 37). Optimum growth of camel *Lactococcus lactis* sub sp lactis strain (pH 6.17) and an optimum production of nisin (IZ= Ø 8mm) were registered for a 24 hours incubation period.

Key words: Bacteriocins, Camel, *Lactococcus lactis* sub sp lactis, Milk, *Staphylococcus aureus*

Introduction

Milk represents about 22% of total food imports in our country. Algeria is thus the second importer of milk and derivatives, after Mexico. It has therefore need every resource in milk, namely that of the goat, sheep and camel particularly adapted to the harsh agro-climatic conditions of the Sahara.

The camel milk has a balanced composition in basic nutrients (proteins, carbohydrates and lipids). This composition is similar to that of bovine milk. It also provides adequate concentrations in minerals and vitamins including vitamin C and niacin, which makes it a nutrient medium favourable to the development of undesirable microbial species such as *Staphylococcus aureus* responsible for mastitis infections and food intoxication.

To defend itself against the development of these microorganisms, camel milk is characterized by a powerful protector system due firstly, to whey protein (lysozyme, lactoferrin, lactoperoxidase, immunoglobulins and component 3 of proteose peptones (PP 3) and secondly to antibacterial substances produced by these lactic acid bacteria (Klaenhammer et al., 1994). These substances include organic acids, oxygen peroxide and bacteriocins purpose of this study.

Materials and Methods

Four samples of camel milk of the Sahrawi population living in extensive in the region of Ouargla were used. They were transported to the microbiology laboratory in a cooler with an ice pack. Bovine milk samples provided by a farm near the laboratory were also used in this study.

The isolation of a lactococci strain was performed on M17 medium and a *Staphylococcus aureus* strain on Chapman's hypersaline medium.

Choice of the target strain

Staphylococcus aureus strain was chosen for:

- sensitivity to bacteriocins (Choi et al., 2000; Allouche et al., 2010) including nisin (Raimbiault, 1995, Sharma et al., 2010);
- belonging to the pathogenic contamination flora in food products (Trias et al., 2008). It is a

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species that can be found in mastitis milk, (Larpent et al., 1997);
- resistance to antibiotics (Rahal, 1984);
- its property "catalase +" to eliminate the inhibitory effect of H_2O_2 in antagonism tests and in mixed cultures;
- its pathogenicity for humans and animals
- its exigency of a selective medium (Chapman medium)

Culture and purification of nisin producing strain and the target strain

Producing strain grew on M17solid medium for 24 to 72 hours at 30°C, the target strain on Chapman medium for 24 h at 37°C.

After incubation, both strains were tested for identification and purified by 4 to 5 subculturing by streak of exhaustion

Propagation of the *Lactococcus lactis sub sp lactis* strain

To prepare a mother -culture, for the production of bacteriocin (nisin), it was preceded to the "propagation of the strain" by the realization of a preculture. This was done by using a protocol reported by Doumandji et al. (2010).

The propagation of the strain consisted in inoculate a colony previously purified from *Lactococcus lactis* (inoculum) in 1 ml of M17 broth and incubated at 30°C for 24 hours: the "intermediate culture 1" was obtained. After incubation, it was proceeded to a second subculture which was transplanted with 1 ml of the previous culture into a tube containing 9 ml of M17 medium: a second culture: "intermediate culture 2" was obtained.

Optimization culture conditions of *Lactococcus lactis subsp lactis*

Supplementation of culture medium with glucose, magnesium, peptone, and yeast extract enhances production of bacteriocins (Heng et al., 2007). Since the - M17 medium contained three types of peptone, yeast extract and magnesium sulfate, only Glucose was added (Heng et al., 2007; Sharma et al., 2010).

Another factor may influence the bacteriocins production. Indeed, Sharma et al. (2010), reported that a maximum growth of *Lactococcus lactis subsp lactis* was observed after 24 h incubation at 35 ° C. Because bacteriocin production is a growth-dependent physiological trait and hence follows primary metabolite kinetics (De Vuyst and Leroy, 2007), two incubation temperatures were used in the present study: 18 hours (usually used) and 24 hours. Two lots were obtained, each lot being divided into two sublots (Table 1).

Table 1. Constitution of the experimental lots of the nisin-producing strain culture.

Lot N°	Sublots	Glucose addition (1%)	Incubation period
1	a	-	18 h
	b	-	24 h
2	a	+	18 h
	b	+	24 h

a: incubation for 18 h **b**: incubation for 24 h

Nisin isolation

After incubation for 24 hours and 18 hours, experimental lots of culture strains were subjected to centrifugation at 8000 r.p.m. (Universal 16 R), for 10 minutes at 4°C.

The resulting supernatant was neutralized to pH 6.5 with NaOH (5N) to remove the antibacterial activity which could be exerted by organic acids (Nykanen et al., 2000).

Study of the antimicrobial activity of bacteriocin from the supernatant

The agar diffusion test by the disc method was performed to investigate the effect of nisin produced by *Lactococcus lactis sub sp lactis* contained in the supernatant on *Staphylococcus aureus* strain.

Results and Discussion

Identification of the strain isolated on M17 and those - isolated on hypersaline Chapman medium

Macroscopic and microscopic examinations and physico-chemical and biochemical tests and biochemical identified the strain of lactic acid producing nisin, as a strain of *Lactococcus lactis subsp lactis* (Plate 1). Other macroscopic and microscopic examination, allowed identifying the target strain, as a strain of *Staphylococcus aureus* (Plate 2).

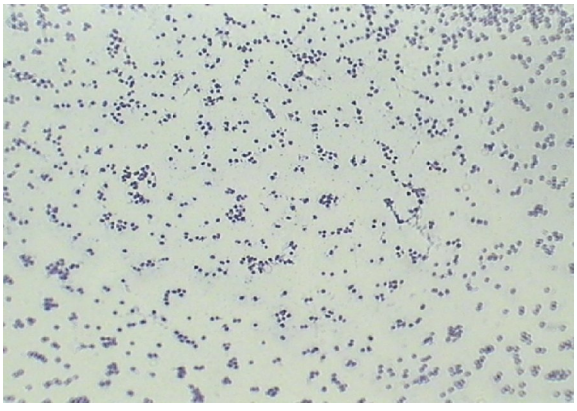


Plate 1. Lactococcus strain after Gram staining (camel milk) (G x 100).

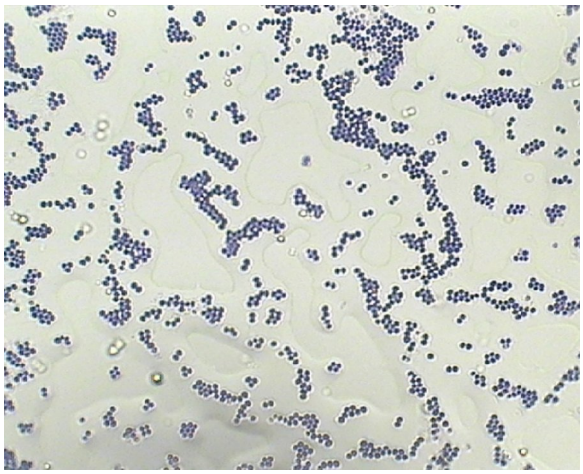


Plate 2. *Staphylococcus aureus* strain after Gram staining (G x 100).

After 4 to 5 successive subcultures of previously isolated strains, pure colonies were obtained.

Culture conditions and optimization of the producing strain

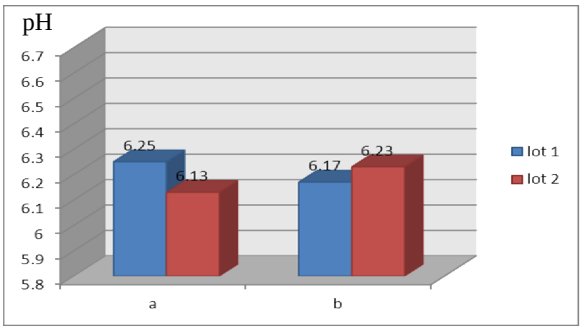
This step was performed with the primary aim to get optimal production of nisin. Bacteriocins production is physiologically dependent on the growth of the producer strain (De Vuyst and Leroy, 2007). This is related to the metabolic activity of the strain, itself linked to the production of lactic acid (Beal et al., 1994) and thus decreasing pH.

Biomass separation

After centrifugation, bacterial cultures and biomass separation, the two lots from supernatants of *Lactococcus lactis subsp lactis* were recovered and the pH measured (Figure 1).

The pH measurement of the supernatant indicated that:

- The glucose addition in a culture of *Lactococcus lactis subsp lactis* incubated for 18 hours increased its growth;
- The extension of the incubation period to 24 hours with the glucose addition had no effect on the growth of the strain;
- the chnage of the incubation period from18 hours to 24 hours of a pure culture of *Lactococcus lactis subsp lactis* enhanced its growth .



a: incubation for 18 hours
b:incubation for 24 hours

Figure 1. pH values of supernatant lots.

Antibacterial activity of supernatants

Tests by the disc method allowed revealing antibacterial activity (Plate 3). Indeed, in all Petri dishes, the IZ appearance was observed. The ZI, recorded in this study was relatively small compared to those reported in the literature. They were between 6 and 8 mm (Keramane, 2009; Doumandji et al., 2010). This result coud be caused by the possibility of partial adsorption of the peptide molecules of nisin on the *Lactococcus lactis subsp lactis* wall and thus its elimination with the pellet (Holo et al., 2002; Rajaram et al., 2010; Sharma et al., 2010). It may also be due to the low concentration of nisin in the supernatant (Holo et al., 2002). Moreover, the diameter of inhibition zones varies with the type of culture medium used and the species used as indicator strain or target strain (Trias et al., 2008).

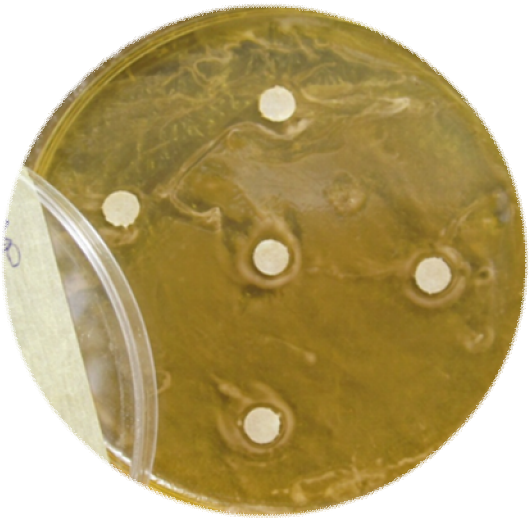


Plate 3. IZ appearance by the disks test.

Nevertheless, the IZ appearance indicated that there was an antibacterial effect of supernatant against *Staphylococcus aureus* strain. Remembered that the antibacterial effect of organic acids, diacetyl and that of H_2O_2, has been discardes by the neutralization of supernatants and by the property of the target strain (catalase +), diacetyl having inhibitory activity against the Gram negative rather than against Gram positive. This means that the appearance of IZ was only due to the production of bacteriocin (type nisin) by *Lactococcus lactis subsp lactis* isolated from camel milk.

For a 24 hours incubation, without glucose addition, better growth (pH 6.17) and increased production of nisin (IZ = Ø 8mm) were recorded.

This result agrees with those of Siboukeur (2007) and Siboukeur and Mati (2008) in which the evolution of the halotolerant contamination flora, during storage at room temperature (30°C on average) has allowed to highlight the appearance of self-purification aspect particularly effective in this milk. The microbiological study undertaken by the same authors showed that the rate of this halotolerant flora decreased during the first three days of storage, while that of lactic acid bacteria tended to increase. This result highlighted the part of bacteriocins in the particular protector system of camel milk.

Conclusion

This study showed that the natural system of camel milk was reinforced by considerable action of nisin produced by *Lactococcus lactis subsp lactis* species. This bacteriocin was particularly effective against one species may accidentally contaminate milk (*Staphylococcus aureus*) and which have developed a resistance to antibiotics according to many authors. It appeared that the synergistic effect of whey protein, organic acid, H_2O_2 and nisin, were responsible for the self-purification effect of camel milk stored, and who keeps it for many hours in relatively high temperatures (about 28°C).

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