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Antimicrobial activity of *Lactobacillus* spp. against *Staphylococcus aureus* ATCC 65 38

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Abstract

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Fermented foods are among the primary source of human nutrition. In Algeria, traditional dairy products are the number one in the family economy. The Jben and Klila cheeses are two of the most popular varieties. The samples of traditional dairy products were collected from the rural area of the Djelfa. Twenty lactic acid bacteria strains were isolated and identified, all belonging to the genus Lactobacillus. Isolates were phenotypically characterized by their capability to ferment different carbohydrates. Among these isolates, four were identified as having a high antibacterial potential against *Staphylococcus aureus* ATCC 65 38. In this study we conclude that species of, Lactobacillus genus isolated from traditional dairy products has been shown to have an antagonistic activities to *Staphylococcus aureus*; this makes it of great importance in the food industry.

Keywords: Traditional dairy products; *Lactobacillus* spp.; *Staphylococcus aureus ATCC 65 38;* antimicrobial activity

Introduction

The consumption of dairy products worldwide is an old tradition since ancient time. These products differ in their taste, denominations and manufacturing processes. Popular dairy products in Algeria are: Zebda (butter), Lben, Imadhghass, Iben, Klila, Bouhezza, Takammart ect. Klila cheese is the most known traditional cheese and produced in different region of this country (Leksir et al., 2019). Some of these products are analysed for physicochemical, microbiological and sensory characteristics. Klila and Bouhezza cheese are investigated for microbial diversity of these products especially of presence for Lactic acid bacteria (LAB), (Ouarda et al., 2011; Guetouache et al., 2015).

Lactic acid bacteria are a diverse group of bacteria that comprise low GC content, Gram-positive bacteria that produce lactic acid, non-sporing, are either bacilli or cocci. These bacteria are wide spread in nature and humans, especially the oral cavity and the human digestive system (Hasannejad et al., 2017). They can be isolated from fermented agricultural products, dairy products, meat products and kefir (Singh et al., 2013; Gulitz et al., 2013). LAB are used in the fermentation and conservation of a variety of dairy products, meats, and vegetables (Bautista-Gallego et

al., 2020). These bacteria are used on a large scale because of their ability to produce a number of antimicrobial substances such as bacteriocins, that might be of importance for food fermentation and preservation; this is what gives it an important economic value (Lozo et al., 2004; Favaro et al., 2015). Bacteriocins, produced by lactic acid bacteria can be defined as small proteins or peptides biologically active, kill directly or inhibit the growth of some bacterial strains from similar or closely related species and usually have a very narrow spectrum of bacterial growth inhibition (Moreno et al., 1999; Hassan et al., 2020). The bacteriocins that are known are classified in three classes: Class I are short peptides such as Nisin A. Class II, Pediocin PA-1, enterocine As-48, ect., are termostabile peptides. Class III comprises larg and heat labile proteins such as helveticins produced by *Lactobacillus helveticus* (Alvarez-Sieiro et al., 2016; Kumariya et al., 2019; Khorshidian et al., 2021).

The studies related to the ability of bacteriocin production by LAB-s have important impact for dairy industry. This is important especially for traditional dairy products, produced by traditional methods which harbor divers' microorganisms including pathogenic microbiome (Quigle et al., 2013). Recently the huge numbers of research are focus in antibacterial effect of LAB-s against pathogenic bacteria, as well as other LAB-s (Yang et al., 2012; Ajazi et al., 2019; Khorshidian et al., 2021). Among others, *Staphylococcus aureus* is a potential pathogenic bacteria found in raw milk and traditional dairy products that can cause foodborn disease. Other studies suggest that LAB such as *Lactococcus lactis* isolated from Algerian cow's milk has been shown to have inhibitory effect against *Staphylococcus aureus* growth (Tetili et al., 2017). The aim of this study was to investigate the antimicrobial activity of *Lactobacillus* spp., isolated from traditional dairy products, against *Staphylococcus aureus* ATCC 65 38.

Materials and Methods

A total of 20 lactic acid bacteria isolates were selected from traditional dairy product. These isolates were identified on the basis of physiological and biochemical characteristics. These isolates were belonging to the genus Lactobacillus (*Lactobacillus fermentum, Lactobacillus intestinalis*, *Lactobacillus plantarum* and *Lactobacillus acidophilus*). The antimicrobial activity of these isolates was detected using the well diffusion method of the indicator isolate *Staphylococcus aureus* ATCC 65 38. Cell free filtrate is obtained by centrifugation with the addition of a 10M solution of NaOH to neutralize the bacterial extract that removes the effect of organic acids. The extract was then filtered on sterile Millipore filters (Guetouache et al., 2015). The *Staphylococcus aureus* were incubated in peptone water at 37°C for 24 h. After 24 h of incubation, 0.1 ml of this culture was inoculated into Petri dishes containing 20 ml of Muller Hinton agar. After planting the pathogen bacteria, the dishes were left for an hour in the refrigerator. Four wells were made and filled with isolated bacterial cell-free filters by incubating Petri dishes at 37°C for 24 h. Then the diameter of the inhibition zone was measured in mm. The antimicrobial activity was determined by measuring the clear zone around the wells (Mathur& Singh, 2005; Saranya & Hemashenpagam, 2011).

Results and Discussion

From total isolates four were selected for further study based on their relatively broad antimicrobial spectrum. The morphological, culturing and physiological characteristics of lactic acid bacteria isolates are shown in Table 1. Selected LAB strains for antibacterial activity assessment were *Lactobacillus fermentum, Lactobacillus intestinalis*, *Lactobacillus plantarum* and *Lactobacillus acidophilus*.

The inhibition zone against *Sataphylococcus aureus* ATCC 65 38, of these 4 isolates were not the same (Figure 1). *Lactobacillus plantarum* in this study had large inhibition zone against indicator bacteria compared to others (Figure 1). *Lactobacillus plantarum* was isolated from different food products in many studies (Ajazi et al., 2018). Other authors were found that the strains of *Staphylococcus aureus* were effectively inhibit by cell free supernatant of *L. plantarum* and this species had sufficient in vitro antibacterial activity against a broad spectrum of foodborne pathogens, including

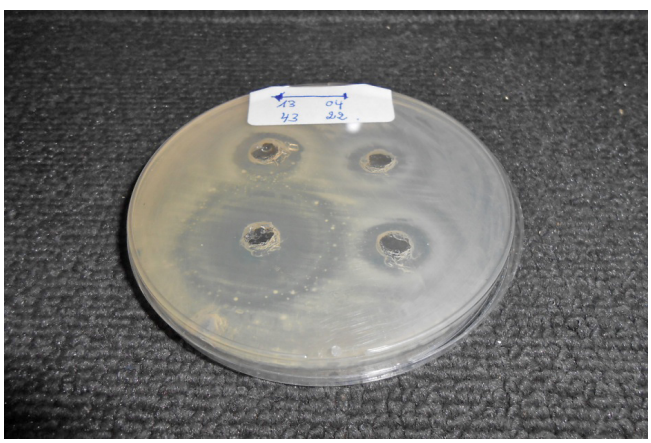

Fig. 1. Antimicrobial activity of *Lactobacillus fermentum (1), Lactobacillus intestinalis* (2), *Lactobacillus plantarum* (3) and *Lactobacillus acidophilus* (4), against *Staphylococcus aureus* ATCC 65 38

Table 1. Morphological, culturing and physiological characteristics of lactic acid bacteria isolates

Species	*Lactobacillus fermentum*	*Lactobacillus intestinalis*	*Lactobacillus plantarium*	*Lactobacillus acidophilus*
Gas from glucose	+	+	–	+
Motility	–	–	–	–
Hydrolysis of:				
– ADH	+	–	–	–
– Citrate	+	+	+	+
Growth at different temperature (°C):				
– 15	–	–	+	–
– 30	+	+	+	+
– 37	+	+	+	+
– 45	+	+	–	+
Growth at different pH:				
– 4.2	–	–	+	+
– 9.6	–	–	–	–
Growth in the presence of NaCl:				
– 4%	–	+	+	+
– 6.5%	–	–	–	–
Sugar fermentation:				
– Arabinose	+	–	–	+
– Cellobiose	+	–	+	+
– Mannitol	–	+	+	–
– Mannose	+	–	+	+
– Melibiose	+	+	+	+
– Raffinose	+	+	+	+
– Ribose	+	–	+	–
– Lactose	+	+	+	+
– Rhamnose	–	–	+	–
– Sorbitol	+	+	+	–
– Xylose	–	–	–	–
– Trehalose	–	–	+	–
– Maltose	+	+	+	+
– Esculin	–	–	+	–
– Sucrose	+	+	+	+

wild strains of *Staphylococcus aureus* (Vatascinova et al., 2020). Also, in other study *Lactobacillus plantarum* CRL 759 was proposed for probiotic application to prevent multi-drug-resistant pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Layus et al., 2020).

Lactobacillus fermentum is known to produce antibacterial peptides which can be used as preserving agent in food industry (Naghmouchi et al., 2020). In this study *Lactobacillus fermentum* was found to be inhibitory for *Staphylococcus aureus* ATCC 65 38 (Figure 1).

Lactobacillus intestinalis is a species of Lactobacillus genus that was shown to have properties that make this as a potential candidate for probiotic use (Jena et al., 2013). Others author found that treatment of rats with a strain of *L. intestinalis* YT2, alleviated menopausal symptoms via the modulation of the gut microbiota and may be consider as a therapeutic- probiotic agent for menopausal women (Lim et al., 2021).

According to many studies is known that strain *Lactobacillus acidophilus* is safe and effective to use for treatment of some disorders of gut microflora in human (Troche et al., 2020). Based on genome sequences some researchers have seen a biotechnological potential of *L. acidophilus* (Huang et al., 2021), and others have suggested using it in the dairy industry to control pathogens, because of its antibacterial activity (Meng et al., 2021).

Conclusion

Traditional dairy products are known to have diverse LAB that is very important for the quality and safety of cheese and other fermented products. In this study *Lactobacillus* species isolated from traditional dairy products had the ability to excrete antagonistic activities against *Staphylococcus aureus* ATCC 65 38, a candidate of pathogenic bacteria. The ability of these LAB species to produce antibacterial

substances against pathogenic bacteria is very important for traditional dairy products produced with raw milk. Based on these results, attention must be given to these bacteria and their exploitation in the food industry, for use as a starter culture in dairy products fermentation. However, further investigations are necessary to research for antimicrobial potential of other LAB against other pathogenic bacteria as indicator and also further study for safe application of these species in food products.

Authors' contribution

Guetouache Moura and Ajazi Flutura C. drafted and prepared the manuscript. All authors had varying roles in collecting data as well as designing and conducting the study.

Conflict of interest: there is no conflict of interest in this study

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