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RESEARCH ARTICLE

IDENTIFICATION OF POTENTIALLY LACTIC ACID BACTERIA FROM DOMIATI CHEESE

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ABSTRACT

Lactic acid bacteria play an important role in a large number of various traditional dairy products. This study investigates isolation and identification of some lactic acid bacteria from Domiate cheese. Six isolates were selected and identified; Domiate cheese isolates had an inhibition and bactericidal effects on the growth of some pathogenic microorganisms as *Staphylococcus aureus*; *E.coli* ATCC 25922 and *Bacillus subtilis* NCIB3610. The isolates had antibacterial effect in deferent degree. *Lactobacillus acidophilus* CH5 isolate was the best effect on the three indicator strains.

INTRODUCTION

Lactic Acid Bacteria (LABs) as a major group of gram positive, catalase negative bacteria are the most important constituent of probiotics and have numerous applications in industry (Salminen and Ouwenhand, 2004). This large group of bacteria includes more than 20 genres to date (Siezen et al., 2002). LABs are mostly microaerophilic and produce lactic acid as their major final product from fermenting carbohydrate (De Vuyst and Leroy, 2009). Although these bacteria are fastidious but are isolated from various sources (Singh and Prakash 2009). Among the most important sources are milk and its products. LABs are responsible for milk aromatic and physiochemical conversion to dairies (Ogier and Gruss, et al., 2002). Because of their high biotechnological potential, isolation and characterization of new strains of LAB for broader industrial application has always been of great importance (Salminen and Ouwenhand 2004). Lactic acid bacteria (LAB) comprise a wide range of genera and include a considerable number of species. These bacteria are the major component of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal micro flora. *Lactobacillus* is one of the most important genera of LAB (Coeuret et al., 2003). LAB strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control biofilm formation and growth of pathogens

(Millette et al., 2006). During the last fifteen years, the *Lactobacillus* genus has evolved and contains to date more than 80 species. They are present in raw milk and dairy products such as cheeses, yoghurts and fermented milks (Coeuret et al., 2003). The major parameters involved in bacterial growth inhibition are the pH, which decreases by the production of organic acid, nutrient competition, hydrogen peroxide and antibiotic production. *S.aureus* is a Gram positive coccus, non – motile non – spore forming facultative anaerobic which appears as grape like clusters. It is a common pathogen associated with hospital acquired diseases which causes major problem for public health. One of the major causes of staphylococcal enterotoxin is vomiting and diarrhea when ingested and is responsible for staphylococcal food poisoning (Nostro et al., 2002). This study was aimed to isolate and identify of some bacterial groups in the Domiate cheese samples from Sadat City, Egypt and evaluation of antibacterial effect of the identified isolates.

MATERIALS AND METHODS

Test organisms

Six isolates were obtained from Domiate cheese and identified as follows: one *Lactobacillus fermentum*; one *Lactobacillus salivarius* and four isolates *Lactobacillus acidophilus*. Isolates were grown in MRS broth at 37°C. The isolates were subcultured every 18 h or 14 h twice before experimental use. Bacterial stock cultures were stored at – 80°C in 15% (v/v) glycerol.

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Table 1. Biochemical identification of isolates

No.	Biochemical tests					API50CH system
	Gram	Catalase	Groth at 10°C	growth at 45°C	production of CO ₂	
CH 1	+	-	-	+	+	99.9% <i>Lactobacillus fermentum</i>
CH 2	+	-	-	+	-	99.9% <i>Lactobacillus salivarius</i>
CH 3	+	-	-	+	-	99.9% <i>Lactobacillus acidophilus</i>
CH4	+	-	-	+	-	99.9% <i>Lactobacillus acidophilus</i>
CH5	+	-	-	+	-	99.9% <i>Lactobacillus acidophilus</i>
CH 6	+	-	-	+	-	99.9% <i>Lactobacillus acidophilus</i>

Table 2. Antimicrobial effect

No.	<i>Bacillus subtilis</i> NCIB3610 mm	<i>E.coli</i> ATCC 25922mm.	<i>Staphylococcus aureus</i> mm
<i>Lactobacillus fermentum</i> CH1	10±1.00	9±1.01	11±2.02
<i>Lactobacillus salivarius</i> CH2	12±0.01	10±0.01	9. ±1.01
<i>Lactobacillus acidophilus</i> CH3	15±0.02	12±1.10	11±0.05
<i>Lactobacillus acidophilus</i> CH4	14±1.05	12±1.03	10±1.02
<i>Lactobacillus acidophilus</i> CH5	19±2.01	15±1.03	13±1.05
<i>Lactobacillus acidophilus</i> CH6	12±1.05	11±1.30	10.5±1.05

Data are presented as mean ±

Identification of isolates

The selection had been done according to Bergey's Manual of Determinative Bacteriology, 9th edition (Holt et al., 1994) with confirm the identification by API 50CH system (Biomerieux, Marcy l'Etoile France).

Study the antibacterial effect

Overnight cultures were previously prepared by inoculated (0.1% v/v) into MRS broth at 37°C for 18 h to until logarithmic phase. The antimicrobial activity was determined according to (Ammor et al., 2006) by measuring the diameter of the inhibition zone around the wells. The bacterial isolates showing the widest zone of inhibition against *Staphylococcus aureus*; *E.coli* ATCC 25922 and *Bacillus subtilis* NCIB3610 were selected for further studies. The experiments were repeated two times in duplicate.

Statistical Analysis

Data are presented as the mean ± standard deviation, and n represents the number of samples from the replicates and the control.

RESULTS AND DISCUSSION

Identification of some lactic acid bacteria

The isolated strain was identified according to methods described in Bergey's Manual of Determinative Bacteriology also, API 50CH system. Genus differentiation of lactic acid bacteria are usually based on Gram staining, catalase test, and determination of carbohydrate utilization using tube or API 50 CHL kit. All isolates were initially tested for Gram stain and catalase enzyme (Harrigan and MacCance, 1976). Only Gram positive bacteria with catalase negative enzyme were observed (Schillinger and Lücke, 1987) and the representative isolates were purified by successive streaking onto the MRS agar. Table 1 showed some characteristics for the six isolates. Identified isolates were confirmed as follows:

One *Lactobacillus fermentum*; one *Lactobacillus salivarius* and four isolate *Lactobacillus acidophilus*. From API CH assay, all of isolate was screened for its performance regarding growth Characteristics using 49 of carbon source and then the results of the analytical data of carbohydrates fermentation were calculated using API software. The result showed that 6 isolates had high % ID (99.9). Isolation of different species of LAB obtained from the same source could be related to the different nutritional status of the isolation sites as observed by Chen et al. (2005)

Antibacterial effect

Well diffusion method was used to identify the nature of inhibitory substances. Table 2 shows the effect of isolated bacteria on the indicator bacteria. There had been a different levels of inhibition zone detected; *Lactobacillus acidophilus* CH5 has the best effect on the three indicator strains forward by *Lactobacillus acidophilus* CH3 and *Lactobacillus acidophilus* CH6; *Lactobacillus acidophilus* CH2 and *Lactobacillus salivarius* CH2 and the lower effect observed by *Lactobacillus fermentum* CH1.

Lactic acid bacteria exert strong antagonistic activity against many microorganisms. This is done through the production of various metabolites such as organic acids, dactyl, hydrogen peroxide and bacteriocin or bactericidal proteins during lactic acid fermentations (Dahl et al., 1989; Vandeborgh, 1993). Besides the production of inhibitory compounds, high numbers of lactic acid bacteria (10^6 cfum⁻¹) compete with the pathogens for nutrients during the fermentation process (Pitt et al., 2000).

Conclusion

Regarding the dairy products, it has been observed that such foods have been greatly explored by industry and scientific researchers due to their health requirements and continuously increasing request by consumers. The antibacterial effect of lactic acid bacteria has been appreciated for more than 10000 years and has enabled man to extend the shelf life of many products through fermentation processes. All the isolated

bacteria tested possess varying degrees of inhibition on pathogenic bacteria.

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