Antimicrobial Effect and Antibiotic Resistance of Lactic Acid Bacteria from some Commercial Dairy Products

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Abstract

Over the past few years lactic acid bacteria (LAB) have received considerable attention as probiotic commonly consumed in fermented foods, such as some yoghurts and fermented milk drinks, and claimed antimicrobial effects against different human pathogens. Therefore, the objective of the study was to investigate the antimicrobial activity of 24 commercial dairy products and the antibiotic resistance in LAB isolated from these products. The antimicrobial effect of commercial products on growth inhibition of *Candida albicans* and *Salmonella enteritidis* was investigated using disc diffusion method, while to test the antibiotic resistance of LAB isolated from dairy were used: ampicillin (SAM, 20 µg), amoxicillin (AMC, 30 µg), ciprofloxacin (CIP, 1 µg) and trimethroprim-sulfamethoxazole (STX, 25 µg). It was found that 8 fermented foods have antifungal activity and the highest effect was produced by Napolact Kefir and a probiotic yoghurt Leeb Vital Bio, but only 2 products (Covalact Sana, Danone Activia) maintained the antimicrobial effect in 7 days expired products. Covalact Sana, Napolact Sana Bio and Pilos (kefir and yoghurt) presented the highest antibacterial effect on *Salmonella* sp. strain. The study revealed high susceptibility of consortium LAB strains from tested dairy products to ampicillin. This preliminary research offers consumers supplementary information about the health benefits of some commercial dairy products.

Keywords: antimicrobial effects, antibiotic resistance, dairy products, lactic acid bacteria (LAB), probiotic.

1. Introduction

Fermented dairy products have played an important role in human nutrition because they confer a health benefit on the host and can be used alternatively for therapeutic purposes in pathogen infections [1, 2]. Many species of lactic acid bacteria (LAB) are involved in the daily manufacturing of dairy products and some of them proved antimicrobial properties against food pathogens or spoilage bacteria and fungi by different mechanisms [3, 4]. Thus, it has been reported that antibacterial effect of yoghurt results from lactic acid produced by LAB cultures used in its production and these products might reduce the negative effects of antibiotics on intestinal microflora [4-6]. Also, bacteria and yeasts that have a symbiotic relationship in kefir have been proved to produce compounds with antimicrobial effect on bacteria such as *Salmonella* spp., *Helicobacter* sp., *E. coli*, *Staphylococcus* sp. and fungus *Candida albicans* [2, 7]. Therefore, it has been recommended for therapeutic purposes that dairy products to be used in foodborne infections associated with antibiotic therapy. However, the optimization of the use of dairy LAB in cases of foodborne diseases requires knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy and to keep the health benefit of LAB on the host. While extensive research has been done on antimicrobial effect of LAB from dairy products, only few papers focused on the antibiotic resistance patterns of these LAB strains [8, 9].

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The aim of this research was to investigate the antimicrobial activity of 24 commercial dairy products on 2 strains (*Candida albicans* and *Salmonella enteritidis*) and the antibiotic resistance pattern of LAB isolated from these products.

2. Materials and methods

Strains and media: The *Candida albicans* and *Salmonella enteritidis* strains from *Applied Microbiology Laboratory*, Faculty of Biotechnology (UASVM Bucharest) was grown on Nutrient Broth Agar and MacConkey Agar media, respectively. The turbidity (OD_{600}) of fungal and bacterial suspensions used in the experiments was 0.43±0.01 and 0.42±0.02, respectively.

Fermented dairy products: In the study, 24 commercial products: probiotic yoghurt, yoghurt, kefir, sana and butter milk were used (*Table 1*). Table 1 shows the pH of fermented products, determined using pH-meter (*Model Basic20+*, *Crison*). In each experiment the samples of product were shaken vigorously to suspend microbial contents before testing.

The 7 days expired products tested were: Covalact Sana, Danone Sana, Danone Activia, Napolact Kefir and Leeb Vital Bio yoghurt.

Lactic acid and hydrogen peroxide solutions: Lactic acid (90%) was diluted with sterile distilled water to 0.9% lactic acid solution with pH = 1.31 (26.5^{0}C) that was adjusted to pH 4.1 (27.7^{0}C), to have the same level of pH as the fermented foods. Hydrogen peroxide 0.3% had a pH of 4.18 (26.6^{0}C).

Disc diffusion method: Discs contained fermented milk product/lactic acid 0.9%/hydrogen peroxide 0.3% were placed on the surface of agar medium inoculated with *C. albicans* or *S. enteritidis* and plates were incubated at 37^{0}C. The diameter of inhibition zones (Θ) were measured at 24 - 48 hours. The antimicrobial effects of 7 days expired products were tested only against *C. albicans*.

Antibiotic resistance of LAB from commercial dairy products: The Kirby-Bauer method was used to test antibiotic resistance to ampicillin (SAM, 20 µg), amoxicillin (AMC, 30 µg), ciprofloxacin (CIP, 1 µg) and trimethroprim-sulfamethoxazole (STX, 25 µg).

Data analysis: The data from 3 experiments with 2 replicates were analyzed by ANOVA ($p<0.05$) and the average values and standard deviation (SD) were reported.

Table 1. The pH values of commercial fermented dairy products tested

No.	Dairy product	pH	SD	T(^{0}C)	SD
1	Covalact Sana	4.26	0.02	24.10	0.70
2	Covalact Yoghurt	4.04	0.01	20.80	3.25
3	Covalact Kefir	4.29	0.01	19.60	4.95
4	Danone Sana	4.06	0.01	20.10	5.52
5	Danone Yoghurt	4.10	0.03	21.97	3.56
6	Danone Activia*	4.16	0.08	23.47	0.50
7	Napolact Sana	4.09	0.14	21.63	4.14
8	Napolact Yoghurt	3.99	0.03	22.43	3.61
9	Napolact Kefir	4.08	0.01	20.60	4.67
10	Napolact Sana Bio	**4.31**	0.01	21.30	4.53
11	Leeb Vital Yoghurt Bio*	3.97	0.05	24.45	0.35
12	Artesana Yoghurt Bifidus*	4.20	0.01	24.60	1.05
13	Cora Yoghurt Bifidus*	4.38	0.02	24.60	0.05
14	Monor Yoghurt Bio	3.96	0.01	24.60	2.45
15	Mugura Butter milk	3.92	0.01	24.60	0.05
16	Mugura Sana	4.25	0.01	24.60	0.05
17	Olympus Bifidus Yoghurt*	4.26	0.25	24.60	0.05
18	Olympus Yoghurt Eco	4.16	0.01	24.60	0.05
19	Panda Butter milk	3.79	0.01	24.60	1.16
20	Pilos Yoghurt Natur	4.06	0.02	24.60	0.05
21	Pilos Kefir	4.11	0.01	24.60	0.05
22	Prodlacta Bifidus Yoghurt*	**3.89**	0.03	24.60	2.05
23	Tudia Butter milk	4.04	0.11	24.60	0.05
24	Tudia Sana	4.09	0.01	24.60	0.75

*probiotic commercial dairy products

3. Results and discussion

A total of 24 commercial dairy products were tested for antimicrobial activity: yoghurt (6), probiotic yoghurt (6), sana (6), kefir (3) and butter milk (3). Among them 5 were organic/bio dairy

products (Table 1). Probiotic yoghurts analyzed in this study contained mainly *Bifidobacterium* sp., but most manufacturers do not offer clear information about the probiotic strains used in the manufacture process of dairy foods. All of the fermented milk products had acidic pH and their pH values vary from 3.89 to 4.38.

In our study, *in vitro* antimicrobial effects of commercial dairy products on fungus *Candida albicans* and bacteria *Salmonella enteritidis* were measured by using the disc diffusion method. The results showed that only 8 commercial fermented dairy from tested products were able to prevent or inhibit the growth of the selected fungus strain (Table 2). LAB strains from 4 yoghurts and probiotic yoghurts and fermented milk drinks (sana and kefir) have antifungal effect on *C. albicans*. The highest activity was noticed with dairy products Napolact kefir and probiotic yoghurt Leeb Vital, that showed zones of inhibition with mean diameters 8 ± 0.2mm and 6.5 ± 05mm, respectively. Similar findings were reported by Rodrigues et al., 2005 who studied the antimicrobial activity of kefir against 7 bacterial species and 1 fungal strain and has been reported that *Candida albicans* was one of the most sensitive pathogen to kefir [3].

Table 2. Antimicrobial activity of commercial dairy products on *Candida albicans*

No.	Dairy Product	Disc method Average Ɵ (mm)	SD
1	Danone Yoghurt	4	± 1.5
2	Danone Activia	4.5	± 1.5
3	Danone Sana	6	± 0.3
4	**Napolact Kefir**	**8**	± 0.2
5	Napolact Yoghurt	3	± 1.0
6	Covalact Sana	4.5	± 0.5
7	Napolact Sana Bio	4	0
8	**Leeb Vital Yoghurt Bio**	**6.5**	± 0.5

However, the dairy foods Napolact kefir and probiotic yoghurt Leeb Vital did not maintained the antifungal activity in 7 days expired products. In our research we tested antifungal activity of 5 products (Covalact Sana. Danone Sana. Danone Activia. Napolact Kefir and Leeb Vital Bio yoghurt) expired for 7 days. The highest effect against fungus pathogen was noticed with expired fermented milk drink Covalact Sana (4.0 ± 1mm) and probiotic Danone Activia (4.5 ± 0.5mm). These dairy fermented milks maintained the antifungal activity almost at the same level in products within the expiration date and expired for 7 days. To our knowledge this is the first report on the antifungal effect of expired dairy products, but additional research data are needed.

Data outlined in Table 3 showed that 8 commercial fermented dairy (3 yoghurts. 3 sana. 1 kefir and 1 butter milk) from tested products have antibacterial activity against *S. enteritidis*. The most effective products were Pilos Bio yoghurt (11.0 ± 0.5mm) and kefir (10.25 ± 1.0mm), as well as sana from Napolact Bio (10.0 ± 0.5mm) and Covalact (10.0 ± 1.0mm). The previous studies with various LAB or with commercial probiotic yoghurts reported the antibacterial effects obtained against *S. typhimurium*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* [1-3].

Table 3. Antimicrobial activity of commercial dairy products on *Salmonella enteritidis*

No.	Dairy Product	Disc method Average Ɵ (mm)	SD
1	Mugura Sana	9.25	±0.3
2	**Pilos Yoghurt Natur**	**11**	±0.5
3	**Pilos Kefir**	**10.25**	±1.0
4	Tudia Butter milk	8	±0.7
5	**Napolact Sana Bio**	**10**	±0.5
6	Napolact Yoghurt	6	± 0.5
7	**Covalact Sana**	**10**	± 1.0
8	Covalact Yoghurt	5	± 1.2

Fermented milk products contain various concentration of lactic acid, 0.9% in kefir and 0.6% in yoghurt [2]. The metabolites produced during lactic fermentation, such as lactic acid or hidrogen peroxide were suggested to induce antibacterial activity [2, 4, 5]. Thus, Yesillik et al. [2] observed that 0.9% lactic acid solution has antibacterial activity against *Pseudomonas aeruginosa*, but the activity disappeared at 48h; while in case of *Salmonella typhimurium* the antimicrobial effect was kept for 7 days [2]. However, in this study, lactic acid and hydrogen peroxide solutions did not inhibited the growth of

bacterial and fungal strains tested. It was concluded that the antimicrobial effects are not caused by some metabolites results during the lactic fermentation. such as lactic acid 0.9% and H_2O_2 0.3%, but are mainly dependent on the type of lactic acid bacteria from fermented dairy products and the tested strains.

The investigation showed different results depending on the type of fermented dairy product used (yoghurt, probiotic yoghurt, sana, kefir and butter milk) and species of pathogen tested (Figure 1). Sana products (Napolact Bio and Covalact) and yoghurt Napolact have both antifungal and antimicrobial activities. Dairy products Leeb Vital Yoghurt Bio and Napolact Kefir with highest activity against *C. albicans* did not created an inhibition zone against *S. enteritidis*. In this study, when inhibition zones are compared, typically, results showed larger inhibition zones on bacteria *Salmonella enteritidis* than on fungus *Candida albicans*. Moreover, commercially probiotic yoghurt tested proved to have antifungal activity more than antibacterial effect. Only 2 bio dairy foods from 5 tested products showed antimicrobial activity.

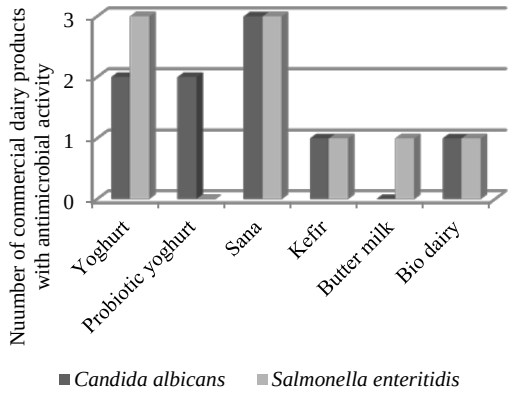

Figure 1. Antimicrobial activity of commercial dairy products according to the type of the fermented food

The antibiotic resistance in the beneficial microbes does not constitute a safety concern in itself, it only becomes a threat when the risk of resistance transfer is present [8. 9]. Currently, it is generally accepted that some probiotic strains with intrinsic antibiotic resistance could be useful for restoring the gut microbiota after antibiotic treatment [8]. Moreover, the optimization of the use of dairy LAB in cases of foodborne diseases requires knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy and to keep the health benefit of LAB on the host.

Investigation on the 13 commercial dairy products that proved antimicrobial effects in our experiments showed 11 different antibiotic resistance profiles of consortium LAB strains from fermented foods (Figure 2). LAB strains from probiotic food Danone Activia and yoghurt Covalact did not created zones of inhibition to all tested antibiotics. Each commercial dairy product showed unique antibiotic resistance pattern induced by the composition of consortium LAB strains from the product. It was noticed that consortium LAB strains from 4 dairy products (Pilos Kefir and Yoghurt Natur. Mugura Sana and Covalact Yoghurt) did not showed antibiotic resistance to all tested antibiotics, while only consortium strains from Napolact sana were resistance to 3 antibiotics and created inhibition zone to CIP (1 µg). Ampicillin (SAM. 20 µg) produced the larger inhibition zone (16.0 ± 0.5mm) on consortium LAB strains from yoghurt Danone. The investigation revealed high susceptibility of LAB strains from tested dairy products to ampicillin (SAM. 20 µg) and amoxicillin (AMC. 30 µg). This is corroborated by data from other groups that tested LAB from some pharmaceutical and dairy products [9-11].

The *Salmonella enteritidis* strain used in this study created inhibition zones to AMC (30 µ) and STX (25 µg).

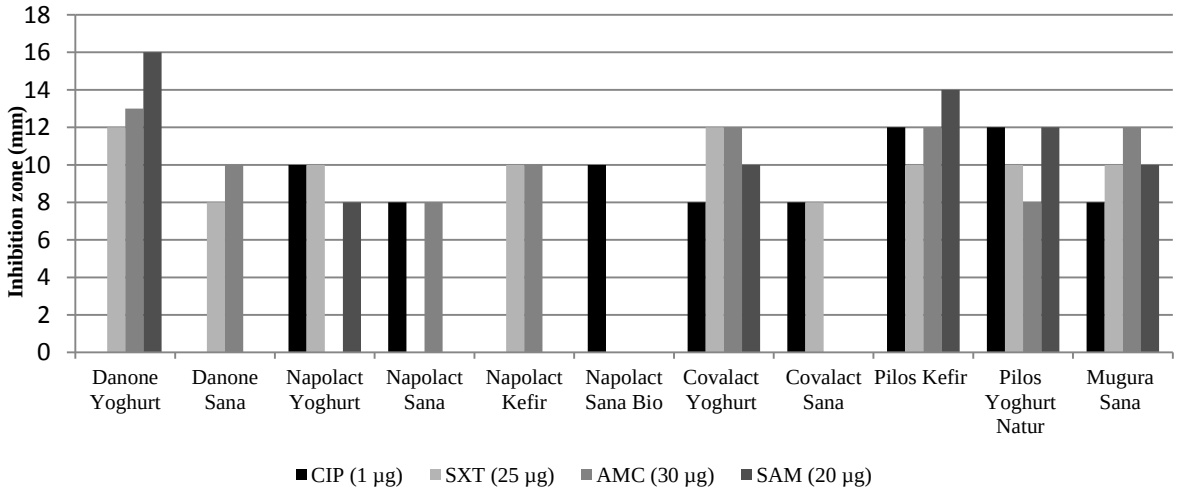

Figure 2. Antibiotic resistance patterns of consortium LAB strains from commercial dairy products

4. Conclusions

In conclusion, presented study showed that some commercial dairy products from 24 tested products have antimicrobial effects against a major opportunistic pathogen fungus *Candida albicans* and a common foodborne pathogen *Salmonella enteritidis*. Different results were obtained depending on the type of fermented dairy product and species of pathogen. Most products did not maintain the antifungal effect in 7 days expired products. Each commercial dairy product showed unique antibiotic resistance pattern influenced by the composition of consortium LAB strains from the product. LAB strains from tested dairy products have high susceptibility to ampicillin (SAM. 20 µg).

This preliminary research offers consumers supplementary information about the health benefits of some commercial dairy products.

Acknowledgements

We acknowledge the continuous support from the UASVM Bucharest.

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