Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

LACTIC ACID BACTERIA INHIBITORY ACTIVITY ON THE PATHOGENS *SALMONELLA* AND *LISTERIA MONOCYTOGENES*

Daniela Sabina Elena VĂTUIU[1], Mona Elena POPA[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +40.734.75.56.03, Email: sabina_bbk@yahoo.com
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +40.745.36.25.76, Email: pandry2002@yahoo.com

Corresponding author email: sabina_bbk@yahoo.com

Abstract

Throughout the technological process of obtaining dairy products occur unintentionally or not, microbial contamination, both internal and external with various pathogens that negative change their quality and threaten the health and safety of consumers. Consumption of dairy products contaminated with pathogens like Salmonella or Listeria monocytogenes causes unwanted effects: food poisoning, meningitis, cardiovascular disease, listeriosis, encephalitis, miscarriage, gastroenteritis, in some cases resulting in death etc.
Using LAB (lactic acid bacteria) in the dairy industry adds a plus protection and safety to consumer by preventing illness; also LAB have a beneficial role on longevity and helps to prolong preservation of dairy products.
Experimental research objectives are intended to level of inhibition by LAB (Delbrueckii subsp. Bulgaricus, Streptococcus thermophilus and Lactococcus subsp. Lactis) on some pathogenic Salmonella and Listeria monocytones twins.

Key words*: LAB, pathogens, degree of inhibition.*

INTRODUCTION

Throughout the technological process of obtaining dairy products microbial contamination could occur unintentionally or not, both internal and external with various pathogens that negatively change their quality and endanger the safety of consumers. Consumption of dairy products contaminated with pathogens like *Salmonella* or *Listeria monocytogenes* causes food illnesses such as: meningitis, cardiovascular disease, listeriosis, encephalitis, miscarriage, gastroenteritis, in some cases resulting in death .
Defined as "microorgansim whose main transmission pathways to humans is through contaminated food during production and processing" (World Health Organization Working Group, 1988), *Listeria monocytogenes* is thought to be the infectious agent that induces the highest mortalityrate. Also, an increasing segment of the population susceptible to infection (due to immune deficiency, malnutrition or aging) makes human listeriosis a very dangerous disease.

Listeria monocytogenes is a psychrotrophic bacteria that grow in food preserved by freezing, prepared and reheated foods - in which is producing listeriolysin; *Listeria monocytogenes* is the causative agent of human listeriosis, a potentially fatal food-borne infection. Clinical manifestations range from fever, gastroenteritis to severe invasive forms including meningitis, encephalitis, abortions and perinatal infections (Dussurget 2008).
Many cases of listeriosis have been associated with the consumption of milk and dairy products in 1980, thus causing a concern for the dairy industry due to increased overall mortality rate by 30%. Following the outbreak alert by *L. monocytogenes* contamination, hygiene measures were strictly enforced, which led to a satisfactory control of the pathogen.
Unhygienic practices cause indirect contamination of milk with *L. monocytogenes* present in the technological flow or feces. *L. monocytogenes* survives during the manufacture and ripening of several types of cheese and is probably to grow if the pH reaches higher values (Gaya et all. 1998).

L. monocytogenes can live as a saprophyte on different natural environments (soil, water, plants, manure, fodder, etc.) or as epiphyte in the body of different animal species. Unlike other non spore forming bacteria it is highly resistant to environmental factors. It resist 12 minutes at 60ºC and 10 minutes at 63ºC. It can be destroyed by the thermal treatments, applied in food technologies. (Bărzoi, 1985).

Lactic acid bacteria (LAB) population able to produce lactose fermentation ensure an acid protective environment by lowering the pH that reaches unfavorable levels for *Listeria* and other pathogens. Organic acids such as lactic acid and acetic acid produced by LAB were found to be considerably more effective as inhibitors of *Listeria* than anorganic acids such as hydrochloric acid (Farber et all., 1989).

Salmonella includes species that are important agents of food poisoning: *Salmonella enteridis, S. dublin, S. Virchow, S. typhymurium* etc. The toxins are intracellular, so its are formed and remain in the cell of the bacterium. After consumption of the product takes place, under the action of HCl in the stomach, bacterial cell are destroyed and the toxin from cells are eliminated. These bacteria can multiply in food but no sensory changes occur. Frequently, contaminated foods are dairy products, chicken, eggs. In gastroenteritis, bacteria multiply in the intestinal lumen and the syndrome occurs after 12 to 24 hours after consumption.

Using LAB in the dairy industry adds a plus of protection and consumer safety by preventing illness, has a beneficial role to the longevity and contributes at extension dairy preservation.

The objectives of the experimental research aimed to determine the degree of inhibition produced by LAB (*L. delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus* and *Lactococcus subsp. lactis*) on *Salmonella* and *Listeria monocytones* pathogens.

MATERIALS AND METHODS

Experiments were performed in SC ICA Research & Development SRL Bucharest - Microbiology Laboratory.

Before starting experiments samples were tested to establish the initial microbial load by lactic acid bacteria. The determination has not confirmed the presence of lactic acid bacteria (*L. delbruecki subsp. bulgaricus*; *S. thermophilus*; *Lactococccus lactis subsp. lactis*) in any samples.

Materials: 36 samples of fresh milk, 42 samples of yogurt, 16 samples of fresh cheese. Samples for analysis were purchased from various local farms.

Equipments: Laboratory instruments, culture media and reagents and reference strains (*Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 13932, Cultures of lactic acid bacteria – *L. delbruecki subsp. bulgaricus*; *S. thermophilus*; *Lactococccus lactis subsp. lactis*)

Methods: SR EN ISO 11290-1/2000/A1/2005, SR EN ISO 6579:2003/AC:2006, ISO 15214:1998, SR EN ISO 6887-1:2002, SR EN ISO 7218:2007, SR CEN ISO/TS 11133-1: 2009, ISO/TS 11133-2:2003, SR CEN ISO/TS 11133-1: 2009

Experiments on the initial microbiological load determination for:

- 36 samples of fresh milk
- 42 samples of yogurt
- 16 samples of fresh cheese

1. Determination of the pH value at the time t_0 (immediately after opening the package).
2. Determination of microbial load on *Salmonella* contamination at the time t_0.
3. Determination of microbial load on *Listeria monocytogenes* contamination at the time t_0.

RESULTS AND DISCUSSIONS

A. Inhibition of pathogen *Salmonella* by lactic acid bacteria

Steps:

o Establishing the nutritional value of the culture medium used in determining *Salmonella* (SVR MKTTn, XLD, BGA)

o Inoculation P1, P2 and P3 with *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and *Lactococccus lactis subsp. lactis.* The samples were inoculated with lactic acid bacterial cultures at 10^8 and *Salmonella typhimurium* ATCC 14028 at 10^6.

- Inoculation P1, P2 and P3 with reference strain of *Salmonella typhimurium* ATCC 14028 (a reference strain inoculation 10^2) (Figure 1)

- Incubation for 24 hours at 37 ^{0}C
- *Salmonella* microbial load determination after incubation at 37^{0}C for 24h
- A replay of determining the microbial load after 48h and 72h.

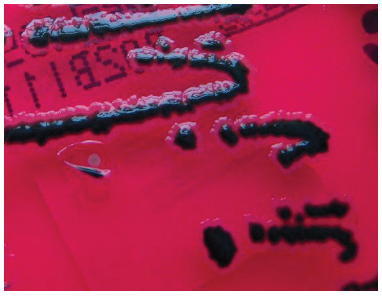


Figure 1. *Salmonella typhimurium* ATCC 14028

Inhibition levels of *Salmonella* by LAB obtained in the experiments carried out on 36 samples of fresh milk (Tabel 1, Figure 2).

Table 1. *L. delbrueckii* subsp. *bulgaricus*

Time of incubation at 37°C (after inoculation *with L. delbrueckii. subsp. bulgaricus*)	Number of samples analyzed	Results
24 h	25	Total inhibition
	11	Partial inhibition
48 h	27	Total inhibition
	9	Partial inhibition
72 h	32	Total inhibition
	4	Partial inhibition

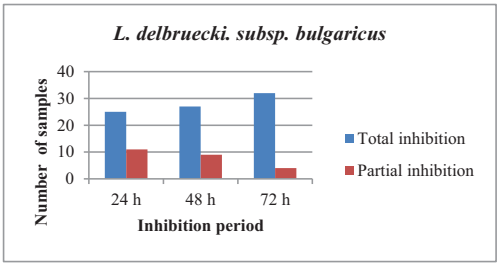


Figure 2. Inhibitory action caused by *L. delbrueckii subsp. Bulgaricus*

Level inhibition on the development of *Salmonella* by LAB obtained in the experiments carried out on 42 samples of yogurt (Tabel 2, Figure 3).

Table 2. *S. termophilus*

Time of incubation at 37°C (after inoculation *with S. thermophilus*)	Number of samples analyzed	Results
24 h	26	Total inhibition
	16	Partial inhibition
48 h	29	Total inhibition
	13	Partial inhibition
72 h	38	Total inhibition
	4	Partial inhibition

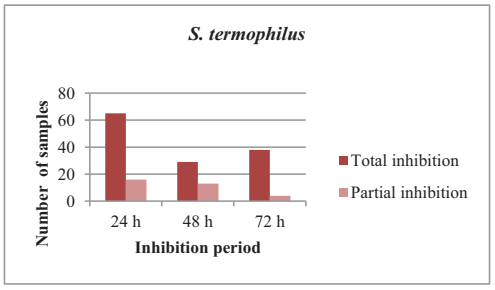


Figure 3. Inhibitory action caused by *S. thermophilus*

Level inhibition on the development of *Salmonella* by LAB obtained in the experiments carried out on 16 samples of cheese (Tabel 3, Figure 4).

Table 3. *Lactococccus lactis subsp. lactis*

Time of incubation at 37°C (after inoculation *with Lactococcus lactis subsp. lactis*)	Number of samples analyzed	Results
24 h	6	Total inhibition
	10	Partial inhibition
48 h	9	Total inhibition
	7	Partial inhibition
72 h	11	Total inhibition
	5	Partial inhibition

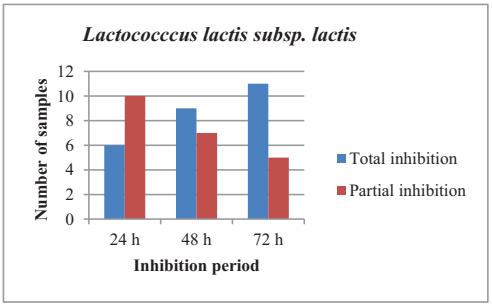


Figure 4. Inhibitory action caused by *Lactococccus lactis subsp. lactis*

B. Inhibition of pathogen *Listeria monocytogenes* by lactic acid bacteria

Steps:

- Establishing the nutritional value of the culture medium used in determining *Listeria monocytogenes* (half Fraser, Fraser, ALOA, Pavement)
- Check *Listeria monocytogenes* ATCC reference strain 13932
- Inoculation P1, P2 and P3 with *L. delbruecki* subsp. *bulgaricus*, *S. thermophilus* and *Lactococccus lactis subsp. lactis* cultures
- Inoculation P1, P2 and P3 with reference strain of *Listeria monocytogenes* ATCC 13932 (a reference strain inoculate 10^2) (Figure 5)
- Incubation for 24 hours at 37 ^{0}C
- Determination of microbial load of *Listeria monocytogenes* after thermostat (24h)
- Repeating the determination of microbes after 48 hours and 72 hours.

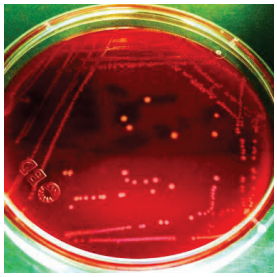


Figure 5. *Listeria monocytogenes* ATCC 13932

Level inhibition on the development of *Listeria monocytogenes* by LAB obtained in the experiments carried out on 36 samples of fresh milk (Tabel 4, Figure 6).

Table 4. *L. delbrueckii subsp. bulgaricus*

Time of incubation at 37°C (after inoculation *with L. delbruecki. subsp. bulgaricus*)	Number of samples analyzed	Results
24 h	22	Total inhibition
	14	Partial inhibition
48 h	28	Total inhibition
	8	Partial inhibition
72 h	31	Total inhibition
	5	Partial inhibition

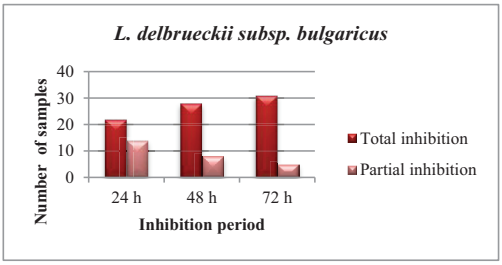


Figure 6. Inhibitory action caused by *L. delbruecki. subsp. bulgaricus*

Level inhibition on the development of *Listeria monocytogenes* by LAB obtained in the experiments carried out on 42 samples of fresh yoghurt (Table 5, Figure 7).

Table 5. *Streptococcus termophilus*

Time of incubation at 37°C (after inoculation *with S. thermophilus culture*)	Number of samples analyzed	Results
24 h	23	Total inhibition
	19	Partial inhibition
48 h	26	Total inhibition
	16	Partial inhibition
72 h	34	Total inhibition
	8	Partial inhibition

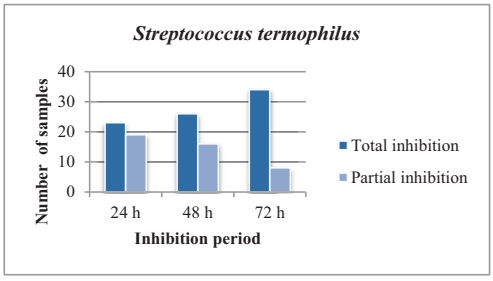


Figure 7. Inhibitory action caused by *S. thermophilus*

Level inhibition on the development of *Listeria monocytogenes* by LAB obtained in the experiments carried out on 16 samples of fresh cheese (Tabel 6, Figure 8).

Tabel 6. *Lactococccus lactis subsp. lactis*

Time of incubation at 37°C (after inoculation *with Lactococccus lactis subsp. Lactis*)	Number of samples analyzed	Results
24 h	9	Total inhibition
	7	Partial inhibition
48 h	11	Total inhibition
	5	Partial inhibition
72 h	14	Total inhibition
	2	Partial inhibition

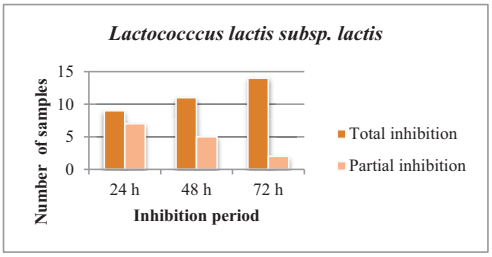


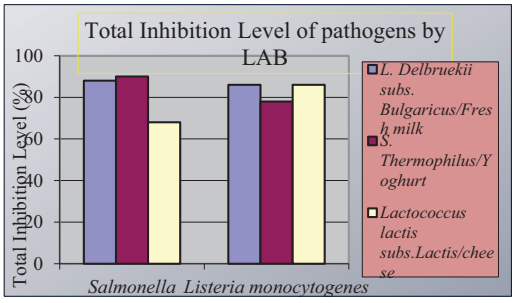
Figure 8. Inhibitory action caused by *Lactococccus lactis subsp. lactis*

CONCLUSIONS

From experiments resulted the following conclusions:

– all types of lactic acid bacteria used in experiments led to the inhibition of the analyzed pathogens - *Listeria monocytogenes* and *Salmonella* - in more than 70% of the samples, after 72h;

– maximum efficiency of inhibition was observed at *L. delbrueckii. subsp. bulgaricus and S. thermophilus* in the case of *Salmonella* and at *L. delbrueckii. subsp. bulgaricus, Lactococccus lactis* in the case of *Listeria monocytogenes*



ACKNOWLEDGEMENTS

This research work was carried out with the support of University of Agronomic Sciences and Veterinary Medicine of Bucharest, in Doctoral School of Engineering and Resource Management Plants and Animals.

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