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

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Abstract

Throughout the technological process of obtaining dairy products occur unintentionally or not, microbial contamination, both internal and external with various pathogens that negatively change their quality and endanger 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 monocytogenes 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 "microorganism 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 mortality rate. 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; S. thermophilus; Lactococcus lactis subsp. lactis) on Salmonella and Listeria monocytogenes 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; Lactococcus 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; Lactococcus lactis subsp. lactis)

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 t0 (immediately after opening the package).
2. Determination of microbial load on Salmonella contamination at the time t0.
3. Determination of microbial load on Listeria monocytogenes contamination at the time t0.

RESULTS AND DISCUSSIONS

A. Inhibition of pathogen Salmonella by lactic acid bacteria

Steps:
- Establishing the nutritional value of the culture medium used in determining Salmonella (SVR MKTTn, XLD, BGA)
- Inoculation P1, P2 and P3 with L. delbrueckii subsp. bulgaricus, S. thermophilus and Lactococcus 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 10^5) (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***

<table>
<thead>
<tr>
<th>Time of incubation at 37(^0)C (after inoculation with <em>L. delbrueckii</em> subsp. <em>bulgaricus</em>)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>25</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>11</td>
<td>Partial inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>9</td>
<td>Partial inhibition</td>
</tr>
</tbody>
</table>

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***

<table>
<thead>
<tr>
<th>Time of incubation at 37(^0)C (after inoculation with <em>S. thermophilus</em>)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>16</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>29</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>13</td>
<td>Partial inhibition</td>
</tr>
</tbody>
</table>

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. *Lactococcus lactis* subsp. *lactis***

<table>
<thead>
<tr>
<th>Time of incubation at 37(^0)C (after inoculation with <em>Lactococcus lactis</em> subsp. <em>lactis</em>)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>6</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>9</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>7</td>
<td>Partial inhibition</td>
</tr>
</tbody>
</table>

Figure 4. Inhibitory action caused by *Lactococcus 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 *Lactococcus lactis* subsp. *lactis* cultures
- Inoculation P1, P2 and P3 with reference strain of *Listeria monocytogenes* ATCC 13932 (a reference strain inoculate 10^3) (Figure 5)
- Incubation for 24 hours at 37 °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](image)

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

<table>
<thead>
<tr>
<th>Time of incubation at 37°C (after inoculation with <em>L. delbruecki. subsp. bulgaricus</em>)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>22</td>
<td>Total inhibition</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Partial inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>28</td>
<td>Total inhibition</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Partial inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>31</td>
<td>Total inhibition</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Partial inhibition</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Time of incubation at 37°C (after inoculation with <em>S. thermophilus</em> culture)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>23</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>19</td>
<td>Partial inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>26</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>16</td>
<td>Partial inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>34</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>8</td>
<td>Partial inhibition</td>
</tr>
</tbody>
</table>

![Table 5. *Streptococcus thermophilus*](image)

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 (Table 6, Figure 8).

![Table 6. *Streptococcus thermophilus*](image)
Tabel 6. *Lactococcus lactis* subsp. *lactis*

<table>
<thead>
<tr>
<th>Time of incubation at 37°C (after inoculation with <em>Lactococcus lactis</em> subsp. <em>lactis</em>)</th>
<th>Number of samples analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>9</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>48 h</td>
<td>11</td>
<td>Total inhibition</td>
</tr>
<tr>
<td>72 h</td>
<td>14</td>
<td>Total inhibition</td>
</tr>
</tbody>
</table>

Figure 8. Inhibitory action caused by *Lactococcus 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*, *Lactococcus lactis* subsp. *lactis* in the case of *Listeria monocytogenes*

ACKNOWLEDGEMENTS

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MISCELLANEOUS