

Microbiology report: ID

Evaluation of NEBUNATURE in the dairy industry.

1. Introduction

Microorganisms present in the air of industrial facilities are involved in numerous processes of food spoilage and represent a potential risk to health. The sanitation of surfaces and environments in the dairy industry is vital for the control of emerging pathogens and spoilage microorganisms such as *Listeria monocytogenes*.

The special ability of *Listeria* for survival and multiplication at low temperatures or environments with high salt concentrations enables its presence in production and processing facilities. This fact has become a complex problem because of its persistence on surfaces and its capacity to form *biofilms*. In addition, its severity and high mortality requires strict hygienic control of facilities and products likely to be contaminated with this organism.

The air sanitizing methodology offers an effective alternative to disinfect surfaces and environments through the use of food additives capable of removing microorganisms without leaving residues that may be harmful to health. Fogging of facilities is effective complementary tool for cleaning and disinfection daily protocols, a new practice that helps to improve the quality of food products.

2. Objet of study

This report shows the results on the microbiological quality of air and surfaces of a dairy factory before and after fogging it with NEBUNATURE (DOMCA SA) in. Analyses were carried out by the Department of Microbiology of DMC Research Center.

3. Experimental design

3.1. Isolation and identification of *Listeria monocytogenes* on surfaces.

For the study of *L. monocytogenes* we did a surface analysis by swab. These sterile swabs (Deltalab, Spain) were previously dipped in the diluting solution and sampled at different hotspots capable of containing bacteria. The procedure was repeated on the same sampling points before and after the cleaning and disinfection of the facilities, and after the fogging treatment. *L. monocytogenes* determination was carried out following the ISO 11290 - 2:2007. It was made a pre-enrichment in Fraser Broth followed by subsequent selective enrichment in Half Fraser Broth (Biokar Diagnostics, France). The phenotypic confirmation was performed on a chromogenic medium for *Listeria* COMPASSLISTERIA (Biokar Diagnostics, France). The culture conditions were 37° C for 24-48 h.

3.2. Environment sampling procedure.

Samples were taken at the facility before and after cleaning and disinfection conventional work (**C + D**). By its completion a treatment with NEBUNATURE by cold spraying was performed. The sampling procedure for evaluating the effectiveness of the product was repeated after a few hours, before the start of the activity. All samples were done in triplicate. The process of sampling is based on agar impaction method (ISO 14-698) by Air Test aerobiocolektor Omega (LCB, France). Then RODAC plates of 55 mm diameter are placed in containing different selected culture media. The volume of air sampled in each unit was 80 L.

Media and culture conditions

For the count of total bacteria in the air a Plate Count Agar (Merck, Germany) was used. Rosa de Bengala Agar (Scharlau, Barcelona, Spain) was used for the determination of moulds and yeasts. The culture conditions were at 30 °C during 24 hours for bacteria and at 25° C during 4 – 5 days for moulds. Once the number of colonies was determined and knowing the air flow and the applied testing time, the results were expressed in colony-forming units per cubic meter of air (CFU/m3).

Expression of results

Once the number of colonies was determined, and knowing the air flow and the applied sampling time, the result was expressed in number of colony forming units per cubic meter of air (CFU/m3). The correction factor associated to the measurement of the sampling device was applied. Air quality of the system was evaluated in reference to the quality parameters mentioned in Table 1.

3.3. Application of NEBUNATURE.

The product **NEBUNATURE** was the one used for the nebulization of facilities. It is a liquid formulation designed for sanitizing air by spraying with food grade active ingredients that hinder the development and proliferation of pathogenic microorganisms, moulds and yeasts. Applications of diluted product in water were performed with the micro-diffuser NEBULIZER DMC. This device produces a homogeneous aerosol with a particle size less than 20 microns, which allows reaching the most inaccessible areas of the facility.

Table 1. Reference environmental quality parameters.

Contamination level	Bacteria CFU / m ³	Moulds and yeasts CFU / m ³
Excellent environmental quality	< 50	< 25
Good environmental quality	50 - 100	25 - 100
Environmental quality to be improved	100 - 500	100 - 500
Contaminated environment	500 – 2.000	500 – 2.000
Highly contaminated environment	> 2.000	> 2.000

4. Results

4.1. Surface analysis

The following table shows the results of analysing surfaces at different locations of the facility.

Table 2. *Listeria monocytogenes* in the facilities.

Sampling points	BEFORE C + D	AFTER C+D	AFTER NEBUNATURE
1 Sink 1	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>	absence
2 Sink 2	<i>L. monocytogenes</i>	absence	absence
3 Transport belt	<i>Listeria spp.</i>	absence	absence
4 Spraying system	absence	absence	absence
5 Salting bath	<i>L. monocytogenes</i>	<i>Listeria spp.</i>	absence

4.2. Microbiological air quality

Results of microbiological quality of air in the workplace and refrigeration chamber, expressed in colony forming units per cubic meter of air sampled, are shown below.

Table 4. Microbiological air quality.		CFU / M ³	
WORKPLACE ROOM	Air volume sample (L)	Bacteria	Moulds and yeasts
1 Before C + D	80	> 2.000	320
2 After C + D	80	> 2.000	650
3 After NEBUNATURE	80	12.5	12.5
REFRIGERATION CHAMBER	Air volume sample (L)	Bacteria	Moulds and yeasts
1 Before C + D	80	> 2.000	225
2 After C + D	80	> 2.000	450
3 After NEBUNATURE	80	0	0

4. Comments

From the data collected in the following report it can be concluded that:

- In the initial analysis three positive results of *Listeria monocytogenes* were identified in the sampling points "salting bath ", "sink 1" and "sink 2".
- After nebulization with the product NEBUNATURE there was no detectein of *Listeria monocytogenes* in any of the places sampled.
- According to the parameters specified in Table 1 and the analysis obtained (Table 4), before the C + D procedures, the contamination of air was high. The works of C + D failed to improve the air quality of the facility. Only after fogging process with NEBUNATURE a significant improvement in the quality of air was achieved, to levels considered as excellent

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