Yersinia ruckeri is the causative agent of enteric red mouth disease (ERM, yersiniosis), one of the most important diseases that affects particularly farmed salmonids species. Numerous articles have demonstrated that Y. ruckeri can cause both epizootics and zoonosis. Y. ruckeri shows the ability to survive outside the host in nutrient-limiting environments for long periods due to biofilms forming capacity with adherence to solid supports but also for the adherence to the host tissues. Considering these aspects, the control of Y. ruckeri can be a problem, because of its resistance. Recently, non-thermal plasma activated water (PAW) proved to be active against Gram-negative bacteria and this fact could be also useful in Y. ruckeri control. The purpose of this in vitro study was to test the antimicrobial efficacy of PAW against Y. ruckeri and to explore the ultrastructural changes in these bacteria. Ultrastructural changes in Y. ruckeri cells, probably related to the action of PAW, included modifications in the shape and texture of the outer membrane. These changes in the bacterial membrane have been linked with the inactivation of bacteria by PAW exposure.

Keywords: AFM, plasma activated-water, Yersinia ruckeri
in order to determine the number of CFU/ml. The plates were incubated at 37°C in aerobic conditions for 24h. The initial concentration of the bacterial suspension determined on Plate Count agar was used as control.

After each contact time, known volumes were transferred onto Löwenstein-Jensen agar in order to evaluate the number of viable bacteria after the PAW treatment.

The reduction of bacterial burden was evaluated using colony-forming unit (CFU) count and the formula: Log Reduction = log10 (CFU before PAW treatment / CFU after PAW treatment).

Also, the experiment was performed on liquid culture media to demonstrated the effect sterilizing of PAW by incubating the type strains treated with PAW on BacT/ALERT bottles.

In order to assess the PAW interactions with bacterial cell wall Dynamic Light Scattering (DLS) and Atomic Force Microscopy (AFM) were used. Zeta potential of the particles were examined on the Delsa Nano Submicron Particle Size Analyzer (Beckman Colter) that uses electrophoretic light scattering (ELS) for zeta potential determination.

To image bacteria by AFM, the same volume of bacteria suspension was deposited on glass cover slips and dried in air at room temperature. AFM images were recorded using an Ntegra Spectra instrument (NT-MDT, Russia) operated in tapping mode under ambient conditions. Silicon cantilever tips (NSG 10) with a resonance frequency of 140–390 kHz, a force constant of 5.5–22.5 N m⁻¹ and tip curvature radius of 10 nm were used.

Results and discussions

The experiment was performed on Yersinia ruckeri RTCC 1877 strain. PAW was obtained in a GlidArc reactor.

Dynamic Light Scattering (DLS) method was used for the Zeta potential assessment. Zeta potential measurements can be used to assess the bacterial surface charge. It is actually a charge on a particle at the shear plane. This value of the surface charge is important for understanding and predicting interactions between particles in suspension. Surface neutralizations of the cell membrane are important for the antimicrobial activity of PAW, which properly acts on the bacterial surface. Timing measurements for the treatment exposure were performed after 1,3,5,7 min of contact with the bacterial suspension of Yersinia. ruckeri. All measurements were done in triplicates. Any positive value on the chart indicates non-viable bacteria (fig. 1 and 2). The zeta potential value strongly correlates with bacteria inactivation (negative cultures) -p < 0.01.

Conclusions

The interaction with PAW agent with the cell surface may involve some mechanisms, interactions with different functional radicals or groups with bacterial surface, resulting in the perturbation of the membrane integrity. This action basically leads to an increase in the cell permeability, which may ultimately result in cell death. Ultrastructural changes in the Y. ruckeri cells were probably related to the action of PAW. These changes included modifications in the shape and texture of the outer membrane as shown in the figure 3. Before the treatment of Y. ruckeri with PAW, the outer surface of the cell was rough, with prominent ridges, but following exposure to PAW, the surface has changed and appeared smoother showing that the periplasmic space has greatly increased after the treatment. Ultrastructural changes in the bacterial membrane mean that the bacteria has been killed by PAW.

The logarithmic reduction was higher than 5 log₁₀ after an exposure time of 2 min proving a powerful bactericidal effect confirmed by positive values of Zeta potential. This
concludes that PAW could be a powerful sterilizing agent for neutralization for the pathogen Yersinia ruckeri.

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