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INFLUENCE OF NITRITE ON THE ULTRASTRUCTURE OF STREPTOCOCCUS FAECALIS

T.Atanasova-Assenova, Meat Technology Institute, Sofia

N.Cherepova, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Nitrite plays a major role in the microbiological safety of cured meat products. The antibacterial effect of nitrite is a well known fact. Enterococci, considered as spoilage microorganisms of cured meats, are very resistant to nitrite./Castellani,(3)/. Bell et al.(2) state that nitrite does not inhibit the aerobic growth of Streptococcus spp., and Yarbrough (10) considers Streptococcus faecalis and Streptococcus lactis as organisms extremely resistant to nitrite and concludes that they are even impermeable to it.

Up to now there is no satisfactory explanation of the molecular mechanisms of nitrite inhibitory action on microorganisms. Data are lacking, as well, on the influence of nitrite on the ultrastructure of microorganisms.

The aim of this study is to follow the ultrastructural shanges in Streptococcus faecalis, cultivated in the presence of nitrite and the role of nitrite in thermal and radiation injury of these microorganisms.

MATERIAL AND METHODS

Organism - Streptococcus faecalis 54-D, obtained from Bulgarian Type Culture Collection

Medium - Plate-count broth, with an addition /after the sterilisation of the medium/ of two nitrite concentrations - 50 and 100 ppm.

Heat treatment - at 70°C for 5 min.

Irradiation - The sediment, obtained by centrfuging the broth culture at 2000 g for 5 min, is suspended in tubes with 2 ml of veronalacetate buffer. The tubes are irradiated on ^{60}Co - source , with a dose rate of 2,4 kGy/h and a temperature during irradiation 0°C. The dose applied is 1 kGy.

Electron-microscopic technique - Kellenberger-Ryter's preparation technique(5) is applied: OsO_4-fixation for 2 h at room temperature; dehydration by alcohols with increasing concentrations; embedding in durcupan by the method of Fluka with a modification of Taxy. Contrasting - by the Raynolds' method. The observations are made on " Opton " CM electron microscope, photographs are taken at 100 kV.

RESULTS AND DISCUSSION

The ultrastructure of stationary phase cells of Streptococcus faecalis /18-h broth cultures/, cultivated in nitrite-free medium /control samples/ is characterized by: A clearly defined three-layer cell wall is observed, comprised of two darker layers /osmiophillic/ - outer and inner, enclosing a wider, homogenous osmiophobic space with a low electron-optical density. The outer layer of the cell wall is smooth and sharply defined /Fig.1- a/. The cytoplasmic membrane is usually attached to the inner layer of the cell wall and it is difficult to distinguish it. The cytoplasm is of uniform granularity because of the uniform distribution of ribosomes./Fig.1- a,b/. In the central part of the cells, as a lighter area, is situated the DNA-carrying organelle i.e the nucleoid./ Fig.1- b/. Because of its low electron-optical density it is clearly distinguished. The nucleoid has a fibrillar-granullar structure, which is presented by dense DNA-fibres, forming a network with a linear arrangement and chromatinic granules, distributed among them, which possess a high osmiophility. In some of the cells, the nucleoid substance is distributed in several zones - a bigger one, and others - smaller, dispersed. /Fig.1 - a/.

When cultivating S.faecalis in nitrite-containing medium / with 50 and 100 ppm/ there some shanges to be noticed in the thin sections. A complex, highly developed intracytoplasmic membrane system /mesosomes/ is to be seen. When nitrite concentration is increased /100 ppm/, mesosomes are to be seen in each of the cells and their number is more than one. There are even cells, the cytoplasma of which is occupied with mesosomes of different structure. The mesosomes are either concentric,round-shaped membranes / Fig.1-d/, or have a tubular structure of shorter and longer tubes, situated in parallel /Fig.1-e/, or resemble myelin figures with membranes, deposed oneover the other,

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Fig.1/a,b,c/. Ultrathin sections of cells of S.faecalis , cultivated in nitrite-free medium /control/; x 190 000.

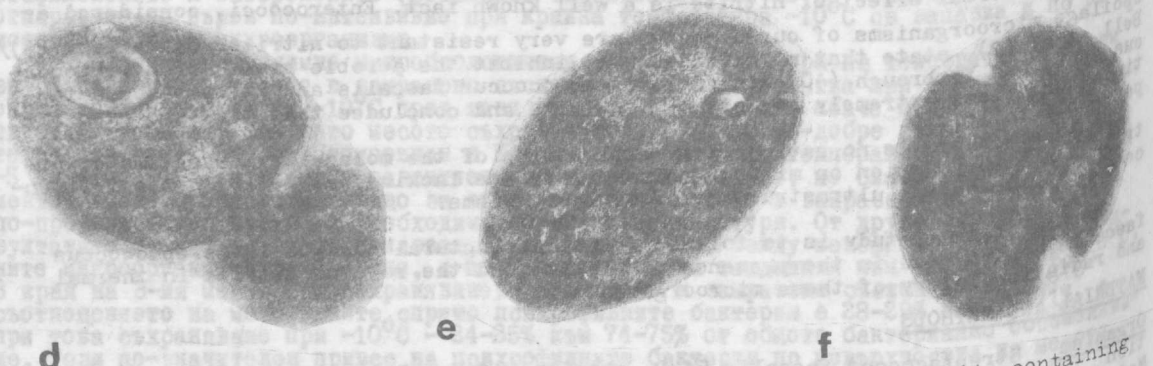

Fig.1. Ultrathin sections of cells of S.faecalis, cultivated in nitrite-containing medium: d/. 100 ppm; x 100 000 . e,f/.100 ppm; x 64 000.

forming many layers. In cells, cultivated in the presence of 50 ppm, a clearly defined nucleoid is observed with its typical fibrillar-granular structure./ Fig.2-a,b/.There are no nucleoid areas observed in cells, cultivated in the presence of the higher nitrite concentration /100 ppm/. The whole space is occupied with ribosomes and mesosomes of different structure/Fig.1- e,f/.

In some of the cells, cultivated in the presence of 100 ppm of nitrite, in some areas a destruction, disintegration and disruption of cell wall and cytoplasmic membrane is observed. In these places the flowing out of cytoplasmic content is observed./Fig.1-d; fig.2-c /.

The exact biochemical functions and physiological importance of bacterial mesosomes are still unclear. There are contradictory ideas about their functional role. Some investigators state that they have no importance for cells' survival and ,consequently, they cannot perform any vital function. Just the opposite - others consider mesosomes as organelles with a specific and necessary biochemical activity./Greenwalt,(4)/.

The influence of nitrite on some enzymatic systems and the metabolitic activities of bacteria has been investigated. Rowe et al.(8) state that nitrite inhibits the active transport, oxygen consumption and oxydative phosphorylation in Pseudomonas aeruginosa. It is possible that nitrite affects these processes on the level of electron transfer in cell membranes. Yarbrough et al.(10) observe a strong inhibition of aldolase in E.coli.Ps.aeruginosa and S.lactis. Meijer et al.(6) state that there is a collapse in proton driving force in Ps.denitrificans as a result of nitrite action / 3 -7 μM of nitrite at pH=7.0/, which is a driving force for ATP-synthesis and other energy-bound processes. The existance of increased proton permeability, when nitrite is present, makes us to believe that nitrite has a direct effect on the cytoplasmic membrane.

The nitrite input level in our investigation /50 and 100 ppm/ is not high, but the ultrastructural changes in S.faecalis show that this exogenic factor corrects and adapts the cells' activity according to the varying conditions of the medium.

Appearance of complex membrane structures is observed after different actions on the cells of Gram-negative bacteria, in which such structures are difficult to be noticed, for example when applying disinfectants, irradiation / Allison,(1)/; heat treatment /Weigand et al.,(9)/ etc.

The phenomena observed allow us to consider mesosomes as "flags, informing about an abnormal state of the membrane"/Greenwalt,(4)/ and, consequently, of the cells, cultivated in the presence of a substance with an inhibitory action towards different bacteria i.e. the sodium nitrite.

It is of interest to know whether there is an interaction between the nitrite and

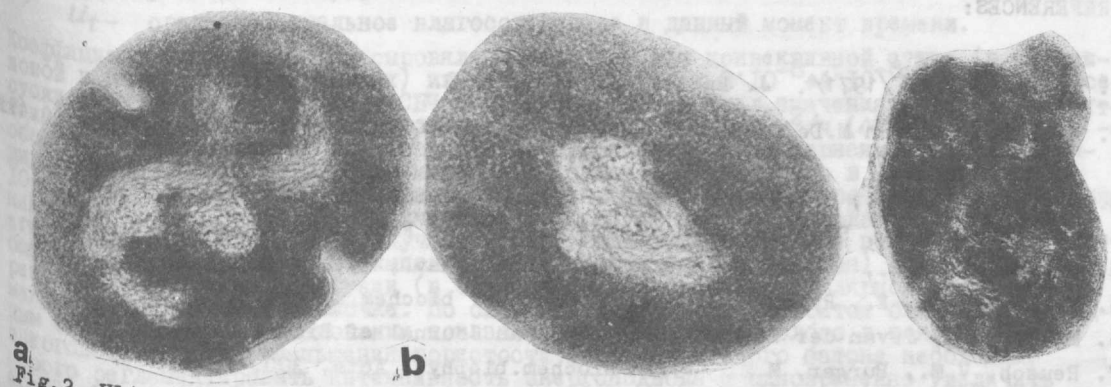

Fig.2. Ultrathin sections of cells of S.faecalis, cultivated in nitrite-containing medium: a,b/. 50 ppm, x 128 000. c/.100 ppm, x 100 000.

Fig.2.Ultrathin sections of cells of S.faecalis, cultivated in nitrite-containing medium /50 ppm/ and combination treatment applied:d,e/ x 100 800.f/x 128 000.

the physical factors applied - heat treatment and irradiation, and what is the role of nitrite in thermal and radiation damage of the cells.

When applying combined treatment to the cells of S.faecalis, i.e. heat treatment and irradiation with a dose of 1 kGy, changes in the structure of cell wall are observed. A very high degree of destruction , disintegration and disruption of cell wall is observed, which is accompanied by flowing out of cytoplasmic content/fig.2-e,f/. A similar effect is in the cells of S.faecalis, cultivated in the presence of 100 ppm of nitrite./fig.1-d/. It is possible, that the changes occurring in the cells, during their growth in nitrite-containing medium, enhance the action of the physical factors applied.

Compact areas in the cytoplasm are observed, where no separate ribosomes can be distinguished. All the ribosomes form a uniform, structureless mass./fig.2-d,e,f/. These changes are due to the action of the high temperature applied, which causes the coagulation of the proteins. Similar compact areas are observed in S.faecalis, cultivated in a medium with 100 ppm of nitrite and applying heat treatment.

In the combination treatment the irradiation dose applied/1 kGy/ does not cause changes, which can be attributed to the action of the γ-rays. A synergistic interaction is observed only between nitrite and heat treatment.

Our observations are confirmed by the findings of Greenberg et al/4a/, who state that there is a synergystic effect of heat treatment and 0,01% of nitrite on the thermal destruction of S.faecium / heat treatment applied at 70°C for 40 min/.

The ultrastructural changes, observed in Streptococcus faecalis, allow us to make the following conclusions:

1. When cultivating the test-microorganisms in nitrite-containing medium the ultrastructure of these organisms undergoes changes. The appearance of highly developed intracytoplasmic membrane system / mesosomes / is connected with the adaptation of cells' behaviour and metabolism according to the varying conditions of the medium.

2. The presence of nitrite in the cultivating medium enhances the action of heat treatment on S.faecalis. It may be concluded that there is a synergistic effect between the action of nitrite and the applied heat treatment.

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