Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series FOOD SCIENCE AND TECHNOLOGY

CEGIELSKA-RADZIEJEWSKA R., LEŚNIEROWSKI G., KIJOWSKI J. 2003. ANTIBACTERIAL ACTIVITY OF LYSOZYME MODIFIED BY THE MEMBRANE TECHNIQUE **Electronic Journal of Polish Agricultural Universities**, Food Science and Technology, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

ANTIBACTERIAL ACTIVITY OF LYSOZYME MODIFIED BY THE MEMBRANE TECHNIQUE

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ABSTRACT

The effectiveness of antibacterial action of lysozyme modified by the membrane technique (ultrafiltration and reverse osmosis) against selected strains of bacteria was determined. Its bacteriostatic activity was dependent on modification conditions. Among lysozyme preparations modified by ultrafiltration the highest bacteriostatic activity against selected strains of *Proteus mirabilis, Pseudomonas fluorescens* and *Staphylococcus epidermidis* bacteria was noted in the preparation containing 53.3% polymeric forms. The modification procedure facilitates the extension of antibacterial spectrum of lysozyme, particularly against *Pseudomonas fluorescens* and *Proteus mirabilis* Gram (-) bacteria.

Key words: lysozyme monomer, lysozyme dimer, ultrafiltration, antibacterial activity

INTRODUCTION

Lysozyme as the enzyme of antibacterial properties has been used both in the pharmaceutical and food industries. Lysozyme additive has been found to extend the storage life of meat and meat products. Particular role plays lysozyme in cheese ageing by the reduction of butyric fermentation bacteria (*Clostridium tyrobutyricum, Clostridium butyricum*), which adversely affect cheese quality.

Lysozyme demonstrates antimicrobial activity against limited spectrum of bacteria and fungi, hovewer its enzyme activity can be enhanced by certain substances including EDTA, butylparaben, tripolyphosphate and as well as by some naturally occurring antymicrobial agents [7]. The lysozyme activity can be enhanced by certain substances and its spectrum against target organisms can be broadened by using chemical synergists or physical treatments that render microorganisms susceptible. It has been found that the spectrum of lysozyme antibacterial action can be broadened by its modification and formation of polymeric forms [1, 2, 7, 9, 11, 18].

The range of lysozyme activity can be extended by modifications leading to changes in the conformation of enzyme molecules and resulting in the production of its polymeric forms. It was found that a modified form of lysozyme shows a significantly wider bacteriostatic activity, including Gram-negative bacteria, than native lysozyme. It has to be emphasized that the modified enzyme retains antibacterial activity against Gram-positive bacteria, characteristic for the monomer [3, 5, 6, 10, 17, 18]. Any modification of lysozyme properties that could render useful in the case of Gram-negative and Gram-positive bacteria would be an important feature.

Lysozyme can be lethal to Gram-negative bacteria if the interaction with the bacterial membrane is strengthened by modifying the enzyme surface hydrophobicity, e.g. chemically modified with palmitate or stearate residues [3, 4] or genetically used with a hydrophobic pentapeptide [5, 8]. All these derivatives exhibit strong bactericidal action against *E.coli* K12. They are capable to insert into lipid layer and subsequently disrupt the electrochemical potential by forming ion pores in the cell membrane. The data suggest that lysozyme molecule can be lethal to bacteria if its structure supplies a hydrophobic domain on the molecule surface.

MATERIALS AND METHODS

Lysozyme obtained from chicken egg white by the ion exchange method [13] developed in the Chair of Poultry Products Technology of the Agricultural University in Poznań was used in experiments. The modification of lysozyme solution by the membrane technique was carried out in the ultrafiltration plate module DDS 20-0.36 LAB at 20Ba pressure, 50°C temperature and pH 7.0, 8.0 and 9.0. Uniq Filtration membranes HR95PP were used. During the modification procedure the thermostat LW102 AURITRONIC kept the temperature at the required level. The obtained lysozyme solutions were spray-dried in Bűchi B-191 Spray Drier [15, 16].

Both the lysozyme monomer and lysozyme preparations modified by ultrafiltration were used in microbiological tests to compare their effectiveness against selected bacteria strains. The details on modified lysozyme preparations used in the microbiological tests are presented in Table 1 where the enzyme activity, the quantities of monomer and polymer forms in the preparations and the modification parameters are specified. The hydrolytic activity was measured using the spectrophotometric method based on the application of the phenomenon of *Micrococcus lysodeickticus* cell wall decomposition by lysozyme [14]. The presence and quantities of the polymeric forms in the enzyme preparations were examined by the electrophoretic technique using SE-600 apparatus of Hoefer Scientific Instruments and the SDS-PAGE method [12, 13].

Gram (+) and Gram (-) bacteria strains: (*Staphylococcus epidermidis ATCC 12228 nr IW 1514, Proteus mirabilis nr IW 513, Pseudomonas fluorescens NCTC 3756 nr IW 1095)* characteristic for poultry meat were used in the experiments. They were delivered by the National Veterinary Research Institute in Puławy (National Collection of Bacterial Strains) and by the Institute of Immunology and Experimental Therapy in Wrocław.

Lysozyme concentrations of 2.5 mg/ml and 5 mg/ml were used. The solution of the required lysozyme concentration was prepared by dissolving the dry lysozyme in sterile distilled water. From 24 h old bacteria culture a series of dilutions was prepared and 1 ml of bacteria suspension was transferred to test tubes with 9 ml of NaCl physiological solution. One ml of bacteria suspension from the respective dilution was added to the test tubes each containing 4 ml of NaCl physiological solution and 5 ml of lysozyme solution. Inoculation of bacteria was carried out onto OXOID culture media: Agar, Mannitol Salt Agar, Pseudomonas Agar Base and MacConkey. Each time lysozyme sterility was examined.

RESULTS AND DISCUSSION

Experiments were conducted with the use of modified membrane technique to obtain lysozyme preparations under optimal modification parameters reported in the earlier studies. Among the examined parameters such as temperature, pH and pressure, the variable parameter was pH (Table 1).

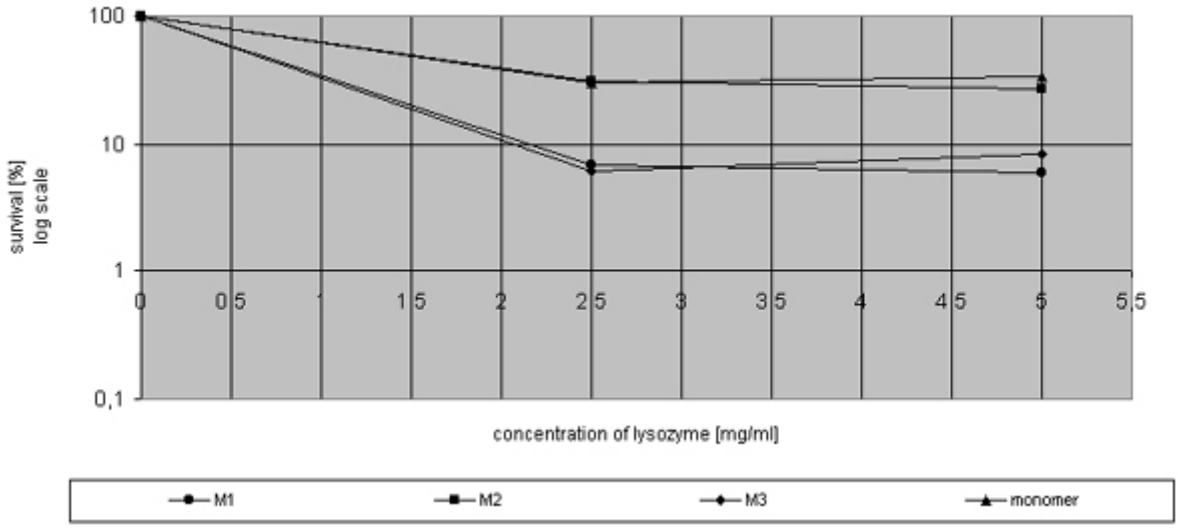
Table 1. Parameters of lysozyme preparations modificated by membrane technique

Lysozyme symbol	Enzyme activity [U/mg białka]	Content of monomer [%]	Content Of dimer [%]	Content of trimer [%]	Modification parameters		
					Temperature [°C]	pH	Pressure [Ba]
M1	6443	46.7	32.7	20.6	50.	7	20
M2	4530	65.5	30.5	6.0	50	8	20
M3	5000	54.9	29.0	16.1	50	9	20

The increase of pH value was found to influence negatively the effectiveness of enzyme polymerisation and at pH 9.0 the lowest quantity of lysozyme dimer was obtained. It was noted that all modified lysozyme preparations demonstrated antibacterial action dependent on kind of bacteria and the lysozyme preparation used.

The tests with *Proteus mirabilis* bacteria concerning the antibacterial action of lysozyme preparations modified by the ultrafiltration technique revealed that M1 lysozyme containing the highest quantity of dimer was most effective (Fig. 1). Similar effective action demonstrated M3 lysozyme preparation. On the other hand, the lowest antibacterial effectiveness in the group of lysozyme preparations modified by ultrafiltration was found in M2 preparation which contained the greatest quantity of monomer and showed the lowest hydrolytic activity. It was also observed that the use of higher concentration of lysozyme had no effect on reduction of *Proteus mirabilis* survival.

Figure 1. The effect of lysozyme preparations on survival of *Proteus mirabilis* bacteria



M1 lysozyme preparation also exhibited particularly effective antibacterial action against *Pseudomonas fluorescent* bacteria. The use of M2 and M3 lysozyme preparations in comparison with the lysozyme monomer also contributed to reduction of *Pseudomonas fluorescent* survival (Fig. 2). The used monomer was found ineffective in the reduction of *Proteus mirabilis and Pseudomonas fluorescent* Gram (-) bacteria number.
The influence of lysozyme preparations, modified by the membrane technique, on S*taphylococcus epidermdis* Gram (+) bacteria showed that M1 enzyme preparation was found most effective similarly as in the case of Gram (-) bacteria (Fig. 3).

Figure 2. The effect of lysozyme preparations on survival of *Pseudomonas fluorescens* bacteria

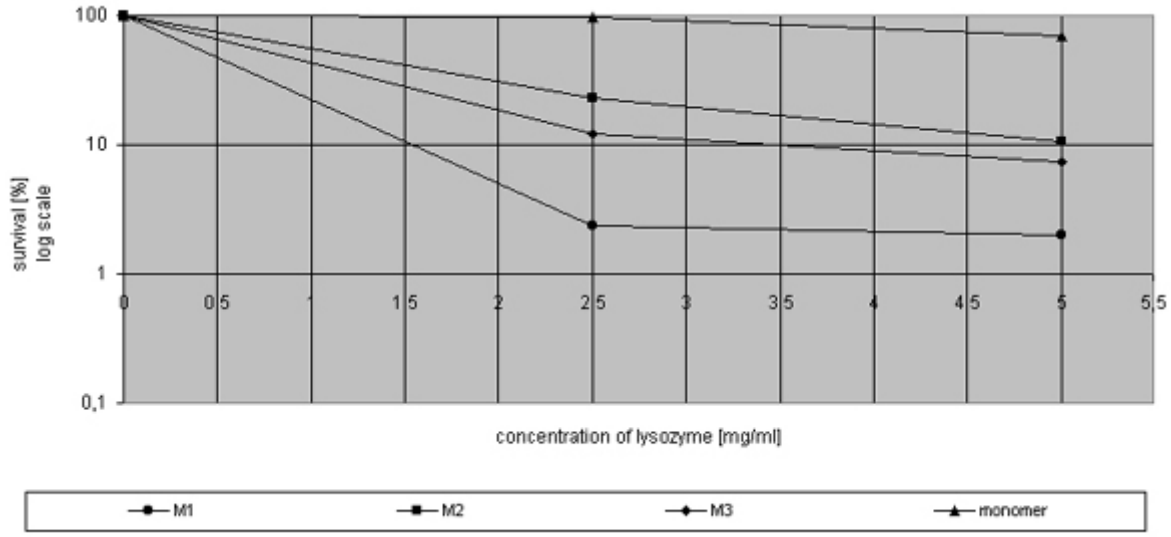
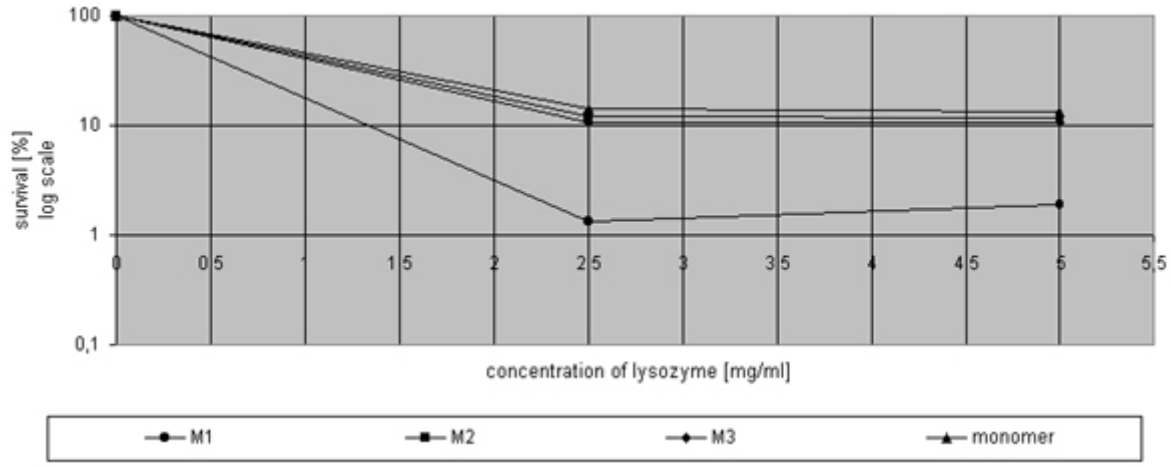


Figure 3. The effect of lysozyme preparations on survival of *Staphylococcus epidermidis*



It was found, however, that lysozyme monomer exhibited substantially greater effectiveness against *Staphylococcus epidermidis* within Gram (+) bacteria in comparison with the other examined bacteria strains of Gram (-) group. On the other hand, a more effective action was observed with some modified lysozyme preparations, e.g. M1. Lysozyme preparations of higher content of M2 and M3 monomer showed antibacterial action similar to that of lysozyme monomer.

Among lysozyme preparations modified by the membrane technique the greatest reduction of the examined bacteria species was noted with M1 preparation which contained the highest quantity of dimer and demonstrated the highest hydrolytic activity (Table 1).

CONCLUSIONS

1. The observed increase in antibacterial activity of modified lysozyme preparations against Gram (-) bacteria was not associated with a decrease of that activity against Gram (+) bacteria.
2. The experiments have shown that the used preparations of lysozyme demonstrate different activity being dependent on the kind of bacteria.
3. Modification of lysozyme by the membrane technique broadened the spectrum of enzyme antibacterial action especially against *Pseudomonas fluorescens* and *Proteus mirabilis*.
4. In the case of Gram (-) bacteria the most effective antibacterial action was noted with lysozyme preparation containing the highest quantity of dimer and the lowest of monomer. It seems justified to investigate the antibacterial activity of lysozyme preparation of low monomer content against a greater number of various bacteria strains.

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This study was financially supported by the KBN grant no. PO6GO3818

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