Antagonistic activity of lactic acid bacteria and extracts from pseudo-fruits and flesh of *Rosa* spp. - as bioactive food ingredients in relation to coagulase-negative strains of *Staphylococcus* spp.

mgr inż. Lidia Piekarska-Radzik

Supervisor: prof. dr hab. liż. Elżbieta Klewicka

Auxiliary supervisor: dr inż. Joanna Milala

ABSTRACT

Staphylococci are microorganisms that, despite their pathogenic nature, are often classified as food contaminants. Many species of the genus *Staphylococcus* are classified as pathogenic bacteria. The consequence of staphylococcal infection can be many diseases, such as: osteomyelitis, endocarditis and phlebitis, meningitis, pneumonia, inflammation of the skin and sepsis. In turn, food contaminating strains are very often associated with food poisoning. Until recently, coagulase-negative staphylococci were thought to be less dangerous than coagulase-posit re species. Nevertheless, bacteria of the genus *Staphylococcus*, which do not show the ability to synthesize coagulase, also pose a serious threat to human and animal health. Among the isolates from the food environment, there are more and more strains classified as multidrug-resistant bacteria (resistance to at least 3 different classes of antibiotics). The increase in antibiotic resistance in the case of coagulase-negative bacteria of the genus *Staphylococcus* necessitates the search for alternative methods of combating these microorganisms.

The research hypothesis was put forward that there is a possibility of limiting the mechanism of pathogenesis of coagulase-negative bacteria of the *Staphylococcus* genus by means of natural methods that can be an alternative to chemically synthesized compounds. Thus, the anti-s' uphylococcal potential of lactic acid bacteria (LAB) and extracts from selected species of *Rosa* spp. (fruit roses) was analyzed.

The first stage of the work involved the isolation and characterization of *Staphylococcus* bacteria isolated from the environment. The matrix for obtaining the strains was food: a dietary supplement, broccoli sprouts and fresh cow's milk (unpasteurized). For the study presented in this paper, 5 environmental strains were selected, whose species affiliation was confirmed using molecular methods using the sequence analysis of the 16S rRNA gene.

Then, the selection of lactic acid bacteria strains, which show a high antagonistic potential towards bacteria of the genus *Staphylococcus*, was carried out. For this purpose, from 50 strains of lactic acid bacteria, 4 strains with the highest anti-staphylococcal potential were selected, presenting species such as: *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Levilactobacillus brevis*, *Lacticaseibacillus rhamnosus*. At the same time, 6 extracts from fruit rose species popular in Poland were obtained: *Rosa canina*, *Rosa rugosa*, *Rosa pomifera*

'Karpatia'. The extracts were prepared from both the whole pseudo-fruit and the fleshy covering of the rue fruit - the pulp.

In the research work, the anti-staphylococcal potential of selected lactic acid bacteria, as well as supernatants obtained after their cultivation, was analyzed. In the supernatants obtained after the culture of lactic acid bacteria, the concentration of lactic acid and acetic acid was determined, as well as the concentration of protein and its antagonistic potential. Thus, it was found that two of the tested strains have the ability to synthesize protein compounds that are characterized by high anti-staphylococcal potential (protein fractions were isolated as a result of ion-exchange chromatography). In this work, the polyphenolic composition of extracts obtained from pseudo-fruits of fruit roses was also determined. The tested extracts effectively inhibited the growth of the tested environmental isolates of the genus *Staphylor accus* (MIC: 3.125 – 500 mg lyophilisate/ml). In the final stage of research on the anti-staphylococcal potential of lactic acid bacteria and *Rosa* spp. extracts, changes in the dynamics of growth or death of the tested strains were determined.

Next, the influence of both factors on the traits associated with the pathogenesis of *Staphylococcus* bacteria was investigated. Changes in autoaggregation, hydrophobicity of cell walls and donor-acceptor properties of cell walls of the tested isolates were determined. Then, the influence of both factors on the ability of the tested bacteria of the *Staphylococcus* genus to form a biofilm (both individually and in a two-component system) was analyzed.

The conducted research on the anti-staphylococcal activity of lactic acid bacteria and extracts from *Rosa* spp. allowed to propose the application of the obtained results. The paper presents two proposals for the industrial use of lactic acid bacteria and extracts rich in polyphenols. The first involved the development of two-component LAB-extract systems that effectively inhibited the growth of staphylococci. The second proposal for the application of the anti-staphylococcal activity of *Rosa* spp. extracts included the development of an innovative cosmetic product - a "greasy base" constituting the basis of a product intended for lip care.

The research presented in this paper verified the positive research hypothesis that lactic acid bacteria and rose pseudofruit extracts have a high antagonistic potential in relation to coagulase-negative bacteria of the genus *Staphylococcus*.

In addition, it was shown that the extracts tested may be an alternative to preservatives in cosmetic products intended for lip care in the fatty system.

Keywords: *Staphylococcus* spp., coagulase-negative staphylococci, *Rosa* spp., lactic acid bacteria, anti-staphylococcal properties