SUMMARY

ISOLATION AND CHARACTERIZATION OF SOME METABOLITES PRODUCED BY LACTIC ACID BACTERIA WITH BIONANOTECHNOLOGICAL IMPORTANCE

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The PhD thesis entitled "***Isolation and Characterization of Some Metabolites Produced by Lactic Acid Bacteria With Bionanotechnological Importance"*** is elaborated by PhD student Iulia – Roxana Ștefan under the supervision of Prof. Univ. Dr. Călina Petruța Cornea within the Doctoral School of Engineering and Management of Vegetable and Animal Resources at the University of Agronomic Sciences and Veterinary Medicine Bucharest, between 2015 – 2019.

Lactic acid bacteria are of great industrial importance, especially in food industry due to the positive effects on food, and on human organism. Living bacterial cultures present on different fermented foods contribute to the improvement of nutritional values and improve the immunity and health of the consumer's intestine. Moreover, the lactic acid bacteria have the ability to preserve food, but also to provide certain fermented products such as cheese and yogurt, unique flavor characteristics. Also, lactic bacteria have many beneficial effects on human and animal health such as immunomodulation, intestinal integrity and resistance to pathogens, which is why they are extensively studied by researchers from a genetic, physiological and metabolic point of view. The enzymatic activity of these bacteria during fermentation contributes to the organoleptic, rheological and nutritional properties of the fermented product. Lactic acid bacteria synthesize numerous important metabolites, including antimicrobial proteins (bacteriocins), cell-surface proteins involved in cellular protection (S-layer proteins) and exopolysaccharides (EPS).

The purpose of this thesis was to isolate and characterize some bio(nano)technological important metabolites produced by lactic acid bacteria present in fresh or fermented foods. Attention was directed to bacteriocins, exopolysaccharides and surface proteins. The key objectives outlined in order to achieve this purpose are:

- Selection of bacterial strains producing the metabolites of interest;
- Characterization of the bacteriocins produced by two bacterial strains belonging to different species and their effect on sensitive strains;
- Isolation and characterization of the EPS produced by two bacterial strains and their impact on the texture of the fermented product;
- Studies on surface proteins synthesized by two lactic acid bacteria.

The present thesis is structured in two main parts: documentation and personal research with a total of six chapters to which was added *Introduction*, *Summary*, *Conclusions* and *Bibliography*.

First part of this work contains three chapter and aims to present the importance of lactic acid bacteria and the metabolites synthesized by them both in the food industry and for the human body consuming functional foods.

The **first chapter** describes the current state of research on metabolites synthesized by lactic bacteria selected for this work: bacteriocins, exopolysaccharides (EPS) and S-layer.

Chapter II highlights the many applications of lactic bacteria and presents the main bacterial families and genres used in the food industry as well as in other fields. Also, this chapter describes the probiotic effects of lactic bacteria and the conditions they must meet in order to be used in probiotic products.

Chapter III is intended to describe the main metabolites synthesized by lactic bacteria, with emphasis on antibacterial compounds (especially bacteriocins), exopolysaccharides, surface proteins, nutraceuticals, vitamins and enzymes.

The **second part** of the paper, the original research, begins with chapter IV in which the purpose of the work and the objectives are presented in detail.

Chapter V includes the methodology used for studies under this thesis. This chapter is structured in five subchapters. The first subchapter describes the culture media used and the bacteria strains producing of metabolites of interest, selected both from the collection of the Microbiology Laboratory of the Institute of Biology, Bucharest, as well as new isolates from different sources. Each subchapter then sums up the methods used to characterize the metabolites. The bacteriocins were characterized in terms of the spectrum of activity and the influence of the culture medium on their synthesis by producing bacteria. After isolation and purification, stability studies were performed at different temperatures, pH values, enzymes, and the detection of coding genes for bacteriocin synthesis was followed. Moreover, in the case of bacteriocin synthesized by *L. lactis* 19.3, its mode of action was studied on two indicator strains.

The study of the exopolysaccharides produced by selected lactic bacteria included isolation and purification, study of their synthesis under various stress conditions, analysis of constituent monomers by TLC and HPLC, effect of culture medium on EPS production and the effect of EPS producing strains on rheological properties of fermented dairy products.

Finally, research on the S-layer included study of its synthesis by the selected bacterial strains grown under different environmental conditions, and its role in protecting the producing cells. The research continued with the detection of the gene responsible for surface protein synthesis by PCR and the study of surface protein synthesis during bacterial growth.

Chapter VI summarizes the results obtained in this PhD thesis and their reporting to other research in the field. The two bacteria with antibacterial activity, *L. lactis* 19.3 and *Lb. helveticus* 34.9 synthesize bacteriocins from two different classes. *L. lactis* synthesizes nisin, a class I bacteriocin, of lantibiotics, characterized by low molecular weight, pH and high temperature resistance. Nisin produced by *L. lactis* 19.3 has a broad spectrum of action, having antibacterial activity on other lactic bacteria and pathogenic or potentially pathogenic bacteria. It acts on the cell wall of the sensitive cells, ultimately leading to their death. Moreover, the bacteriocin synthesis also occurs under various stress conditions, including in acidic media or in the presence of bile salts, which may recommend the producing strain for a probiotic product. On the other hand, *Lb. helveticus* 34.9 synthesizes helveticin, a class III bacteriocin, characterized by high molecular weight, thermolabile, stable to pH variation and inactivated by proteinase K. This bacteriocin was distinguished by antibacterial activity against the *Halobacillus hunanensis* 5Hum strain, responsible for the deterioration of murals in Humor monastery.

The EPS synthesized by the two selected bacterial strains are of large size, are made up of glucose and are synthesized under both optimum and stress conditions, depending on the strain. The viscosity of cow's and soybean milk fermented with the two EPS-producing strains was variable, and its value cannot be correlated in all cases with the amount of polymer, possibly being also influenced by the specific properties of EPS.

The two S-layer producing strains selected for this work are capable of producing these proteins under both optimal and stress conditions. Maximum S-layer synthesis by *Lb. helveticus* 34.9 takes place at 37 °C (optimum temperature) followed by cultivation at 42° C, then the other cultivation conditions. On the other hand, in the case of strain *Lb. brevis* FV 403, S-layer synthesis is more intense under stress conditions (cultivation at 20° C, followed by the addition of sodium chloride in growth medium or low initial pH) than under optimal conditions of cultivation. The S-layer proteins have a protective role over the producing bacterial cell and the removal of this coating by treatment with

LiCl 5M increases cell sensitivity and mortality, especially in media with acidic pH or in the presence of bile salts. The amplification with primers for the S-layer encoding gene (*slp*A) resulted into obtaining gene-specific reaction products with appropriate molecular weight for both tested strains. It was observed that S-layer proteins are present throughout the growth of *Lb. helveticus* 34.9 strain, under optimal conditions, more intense protein bands being observed in the exponential growth phase, suggesting the protective role of S-layer during cell division.

Finally, the conclusions highlight the main characteristics of the metabolites synthesized by the bacterial strains selected for this work. The data presented may contribute to the development of new food or industrial products with improved properties.