Summary of Professional Accomplishments

Appendix 3

Dorota Zielińska, Ph.D.

Warszawa, 2019

1. Personal details and professional experience

1.1. Name and Surname

Dorota Zielińska

2. Diplomas and scientific degrees

17.12.2008: **Doctor of Philosophy,** discipline: food and nutrition technology, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences,

- Thesis topic: Predictive models of survival of probiotic bacteria in fermented soy beverage

07. 08. 2003: **Master of Science;** discipline: food and nutrition technology, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences,

- Thesis topic: Survival of the probiotic strain *Lactobacillus acidophilus* in vegetable juice

3. Information on employment in research institution

15.12.2010-present:

Assistant professor; Researcher and lecturer, Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumer Sciences, Department of Food Gastronomy and Food Hygiene

01.03.2010-30.09.2012

Secretary of the Interdepartmental College of Commodity Science, Warsaw University of Life Sciences

15.12.2008-14.12.2010

Research assistant; Researcher and lecturer, Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumer Sciences, Department of Food Gastronomy and Food Hygiene

Furthermore:

01.09.2003-14.12.2008

Ph.D. student; Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumer Sciences, Department of Food Gastronomy and Food Hygiene

01.09.2003-14.12.2008

Teacher of vocational subjects at the Gastronomic Schools Complex E. Pijanowski, Warsaw, Poznańska st. 6/8

4. Main Scientific Achevement

4.1.Title of the scientific achievement:

The scientific achievement, in accordance with Article 16, Paragraph 2 of the Act of 14 March 2003 concerning the scientific degrees and titles (Journal of Laws No. 65, item 595, as amended), is the series of publications entitled:

Functional and technological properties of lactic acid bacteria strains isolated from food, determining their probiotic effect

4.2.The list of publications constituting the scientific achievement

H1. Zielińska D., Rzepkowska A., Radawska A., Zieliński K. (2015): *In vitro* screening of selected probiotic properties of *Lactobacillus* strains isolated from traditional fermented cabbage and cucumber, *Current Microbiology*, 70, 2, 183-194.

IF_{2015}= 1,519; $MNiSW_{2015}$ = 15 pkt., the number of citations WoS = 15

H2. Klindt-Toldam S., Larsen S.K., Saaby L., Olsen L.R., Svenstrup G., Müllertz A., Knøchel S., Heimdal H., Nielsen D.S., **Zielińska D.:** Survival of *Lactobacillus acidophilus* NCFM® and *Bifidobacterium lactis* HN019 encapsulated in chocolate during *in vitro* simulated passage of the upper gastrointestinal tract. *LWT - Food Science and Technology*, (2016) 74, 404-410.

IF_{2016}= 2,329; $MNiSW_{2016}$ = 35 pkt., the number of citations WoS = 8

H3. Zielińska D., Długosz E., Zawistowska-Deniziak A. (2018): Functional properties of food-origin *Lactobacillus* in the gastro-intestinal ecosystem - *in vitro* study. *Probiotics and Antimicrobial Proteins*, https://doi.org/10.1007/s12602-018-9458-z

IF_{2018}= 2,345; $MNiSW_{2017}$ = 20 pkt., the number of citations WoS = 1

H4. Mituniewicz–Małek, A., **Zielińska, D**., Ziarno, M. (2019): Probiotic monocultures in fermented goat milk beverages–sensory quality of final product. *International Journal of Dairy Technology*. https://doi.org/10.1111/1471-0307.12576

IF_{2018}= 1,225; $MNiSW_{2017}$ = 20 pkt., the number of citations WoS = 0

H5. Zielińska D., Kołożyn-Krajewska D. (2018): Food-origin lactic acid bacteria may exhibit probiotic properties: review. *BioMed Research International*, Article ID 5063185, 15 pages.

IF_{2018}= 2,583; $MNiSW_{2017}$ = 25 pkt., the number of citations WoS = 0

The total score of all works constituting the Main Scientific Achievements is **115 points** acc. to MNiSW journals' rank and total **IF =10.001** (IF from the year of publishing), **sum of number of citations WoS = 24**. In all publications I am the corresponding author. Copies of manuscripts included in the monograph constituting the Main Scientific Achievement together with declarations of co-authors concerning their contribution to each of these manuscripts are enclosed in Appendix 5.

4.3. Presentation research objective and results obtained within the Main Scientific Achievement and the presentation of their possible use

Introduction

One of the fastest growing and most promising areas of science development in the last two decades in the field of human nutrition is the use of probiotics and determining their impact on human health. Lactic acid bacteria (LAB) are most often used as probiotics in conventional foods and in food supplements. The mechanisms of action of probiotic bacteria that affect human health are not fully explained, but may include competition against intestinal pathogens, the decomposition of carcinogens and anti-nutrients, production of antimicrobial metabolites and modulation of the immune response of the gastrointestinal mucosa (Magalhães i wsp., 2018).

In 2002, FAO and WHO experts adopted the definition of probiotics, recognizing that they are: "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*". Microorganisms, in order to be considered as probiotic, should be taxonomically defined and meet specific criteria regarding safety, functional and technological characteristics. The key requirements for probiotics are:

1) must be alive when administered to the body and must be microorganisms;

2) must be given in an appropriate dose, closely related to clinical documentation;

3) must have a beneficial effect on the host (Sanders, 2014).

In 2014, ISAPP experts (International Scientific Association for Probiotics and Prebiotics) organized a meeting, the result of which was a publication verifying the previous FAO / WHO Report (2002). A consensus was announced that included minor grammatical corrections to the definition, leaving it meaningful. Probiotics can be called many types of microorganisms that show health benefits for the host while remaining alive. The presented document particularly underlined this feature and excluded from the definition of "probiotic" metabolites and dead cells of microorganisms. In addition, it was agreed that "probiotics" are not undefined consortia of microorganisms (such as fecal microbiota transplants) and fermented foods containing undefined microorganisms (eg sauerkraut) (Hill et al., 2014).

Due to the fact that probiotics must have a beneficial effect on the host, it is believed that they should come from the healthy human intestinal environment, and thus exhibit resistance to digestive enzymes, low pH, high concentration of bile salts (Guarner et al., 2005). It is also recommended that in supplements and food the probiotic strains

used should be isolated from the population in which they are to be applied later (Nowak et al., 2010). However, it seems that the specificity of action, not the source of isolation of the microorganism, is important. Most of the probiotic strains used in humans have been isolated from humans, but this recommendation is not a requirement. There are known are several well-studied non-human probiotic strains (e.g., *Bifidobacterium animalis* subsp. *lactis* and *Saccharomyces cerevisiae* var. *boulardii*). In fact, it is very difficult to confirm the origin of the microorganism (Sanders, 2014).

Isolation, identification and assessment of the safety and probiotic properties of new strains of microorganisms is a necessary practice, especially nowadays when progressive loss of biodiversity of human microbiome is observed (Blaser, 2016). Efficiently functioning intestinal ecosystem, so-called microbes, has a big impact on the preservation of human health. Due to the growing awareness of the role of intestinal microflora for human health, for more than 20 years, research has been carried out around the world related to the possibilities of positive modification or enrichment of the human microbiome. It is believed that the use of appropriately selected probiotic bacterial strains in nutrition may favorably modulate the intestinal microbial composition (Kerry et al., 2018).

Newly isolated and characterized bacterial strains can be used in the further stages of research to construct innovative starter cultures dedicated to food. In addition to the protective properties (bacteriostatic and bactericidal), the new starter cultures may add additional values related to the improvement of consumer health by modeling the composition of the product, increasing the availability of bioactive components, as well as probiotic activity. What's more, it has been proven that food ingredients, or an appropriate matrix, can protect bacteria and facilitate their survival in difficult conditions prevailing in the human gastrointestinal tract (Fontana et al., 2013, Neffe et al., 2018).

Therefore, bacteria with potential probiotic features beside the conventional source (healthy human digestive tract) may come from unconventional sources such as animal digestive tract, female milk, food (fermented and unfermented), air or soil, as suggested by some studies (Fontana et al. , 2013, Ren et al., 2014, Vinderola et al., 2017). According to the current state of knowledge, probiotic microorganisms should show the effect of improving the health of the host. The origin of microorganisms from the human gastrointestinal tract is not a criterion indicated as necessary, although many sources state differently (Salminen et al., 1998, Collins and Gibson, 1999). The more so because more and more scientific evidence points to new unconventional sources of isolation as appropriate. Furthermore, microorganisms isolated from food often show better survival in the food environment and guarantee more attractive sensory characteristics of fermented food compared to intestinal microbes, when added (Fontana et al., 2013, Neffe et al., 2018).

The properties of many strains of lactic acid bacteria isolated from unconventional sources are consistent with the recommendations of the FAO / WHO regarding probiotics, except one - they do not come from the human gastrointestinal

tract. In my research, presented as a scientific achievement, I have posed the fundamental question: for many new scientific evidence, should there be a possibility to change the current FAO / WHO requirements for the definition of probiotic bacteria and to emphasize the importance of the source of isolation?

The aim of achieving, being the basis for applying for the academic post-doctoral degree (habilitation) is the characteristics of selected functional and technological properties of *Lactobacillus* bacteria isolated from fermented food and the assessment whether the presented properties allow to classify the strain to probiotic bacteria.

The scope of research included:

- Isolation, identification and initial selection of *Lactobacillus* strains in terms of safety.
- Evaluation of the survival of selected *Lactobacillus* bacteria under *in vitro* conditions simulating the gastrointestinal passage and analysis of factors determining resistance to these conditions.
- Analysis and evaluation *in vitro* of selected probiotic properties chosen *Lactobacillus* strains.
- Assessment of the applicability of selected strains of *Lactobacillus* in order to shape the quality of functional foods.

Research hypotheses:

1. It is possible to isolate lactic acid bacteria from traditionally fermented food and to select safe strains when used in humans, sensitive to antibiotics and not producing undesired enzymes.
2. The lactic acid bacterial strains isolated from traditionally fermented foods are resistant to the conditions prevailing in the model gastrointestinal tract. The survival of probiotic bacteria under conditions simulating the stomach environment is influenced by the type of food carrier as well as the dose of bacteria used.
3. *Lactobacillus* bacterial strains isolated from traditionally fermented foods have the ability to adhere to intestinal epithelial cells in vitro and immunoregulatory properties.
4. The possibility of using *Lactobacillus* bacteria isolated from traditionally fermented foods as starter cultures for the production of functional products is diverse and conditioned by, among others, receiving products with acceptable sensory features.

The results and discussion

Isolation, identification and initial selection of *Lactobacillus* strains, in terms of safety

In publications H1 and H5 I undertook issues of isolation, identification and safety of LAB isolated from fermented food.

Food fermented in a traditional manner, which consisted of pickled cucumbers and cabbage, originating from independent households, from various regions of Poland, were the source of isolation of lactic acid bacteria (H1). A total of 38 strains were isolated from the collected samples, of which 17 were classified to the genus *Lactobacillus* sp. based on the appearance of the cells under the microscope, Gram staining, catalase test, as well as growth at 10, 15 and 45 ° C and growth under pH conditions. = 3.9 and 9.6. Further identification was based on isolation of bacterial DNA, amplification of 16S rDNA fragments using universal primers 27F / 1492R (5'-AGA GTT TGA TCC TGG CTC AG-3 '/ 5'-GGT TAC CTT GTT ACG ACT T-3') and sequencing PCR products. Based on the obtained nucleotide sequences, 14 strains of the tested *Lactobacillus* bacteria were identified, assigning them to the species: *casei, plantarum, brevis, rhamnosus, johnsonii*. Comparing the analysis of identification using genetic methods with identification using biochemical methods, it was found that the results are not convergent in all cases. According to FAO / WHO recommendations, for full identification it is necessary to sequence genetically conserved sections, i.e. 16S rDNA or 23S rDNA. Proper identification of bacteria to be used as probiotics is a very important element, because the functional characteristics of the bacteria are strain-dependent and can not be extrapolated to a species or genus. **I found that the identification only using biochemical methods is insufficient and may give false results. However, the biochemical activity profile may be helpful in identifying technologically advantageous enzymes produced by bacteria, such as β-galactosidase, or exclude microorganisms that produce harmful enzymes, such as β-glucuronidase, α-chymotrypsin, β-glucosidase, or N-acetylated enzymes. β-glucosaminidase.** These harmful enzymes may generate potentially carcinogenic metabolites in the colon (Heavey, Rowland, 2004). Based on the experiments carried out, three strains, that produced undesired enzymes were excluded from the pool of test strains.

The safety of using probiotics in humans is associated not only with the possibility of producing undesirable enzymes, but also with the problem of antibiotic resistance. As a result of the experiments conducted as part of the research presented in the H1 publication, it was found that the majority of *Lactobacillus* strains tested, isolated from fermented foods, are sensitive to test antibiotics. Only two strains from the 14 subjects were excluded from further studies due to resistance to tetracycline, which can be transmitted by plasmids, which is associated with the risk of increasing antibiotic resistance. This thesis, however, should be verified in the further research. The *in vitro* diagnostic tests proposed and used by me in the H1 publication allow quick and cheap

pre-selection of strains in terms of safety. **Finally, as a result of research (H1) related to isolation, identification and initial safety assessment of *Lactobacillus* strains, for further studies on the identification and characterization of probiotic properties, I selected 10 strains: *Lb. casei* (O12, O13, O16, O18), *Lb. plantarum* (O19, O20), *Lb. brevis* (O22, O24), *Lb. rhamnosus* K3 and *Lb. johnsonii* K4.**

Preliminary results of the research presented in the H1 publication prompted me to deepen the knowledge about various sources of bacterial isolation with probiotic properties. As a result of the search, I noticed that the lactic acid bacteria with probiotic features, were isolated from various conventional sources (gastrointestinal tract of a healthy person), as well as unconventional, such as: animal digestive tract, fermented and unfermented food, food waste, sewage, soil or air. In the H5 publication, a number of examples of studies on probiotic properties, including antimicrobial LAB strains isolated from conventional but above all unconventional sources, have been collected and cited. Other, not included in this scientific achievement, publication are also mentioned as examples. In publication H5 I used my own experience and observations, which show that strains of bacteria isolated from Polish food, ie regional cheeses, organic whey, fermented vegetables and meat products, show probiotic features. When analyzing data from own studies, it was noticed that antimicrobial properties are dependent and characteristic for the source of isolation. For example, LAB strains isolated from "oscypek" mountain cheeses were characterized by strong anti-*Listeria monocytogenes* properties, whereas LAB strains isolated from Korycin cheeses represented anti-*E. coli* (H5) properties.

An important achievement and conclusion resulting from the analyzes presented in the H5 publication is to demonstrate the need to isolate and identify new LAB strains and their characteristics in terms of safety and functionality. Strains obtained from local sources, given in a high dose, could support and increase biodiversity in populations of human gastrointestinal microbiota, improve health and fitness, and find use as natural bioconservatives in food production.

On the basis of own research presented in H1 and based on literature analysis, which was made in publication H5, the first hypothesis was positively verified, showing that traditional and regional fermented food is a rich source of lactic acid bacteria, among which are strains represented antimicrobial propoerties, safe for human use.

Evaluation of the survival of selected Lactobacillus bacteria under in vitro conditions simulating the gastrointestinal passage and analysis of factors determining resistance to these conditions.

In publications H1, H2, H3 and H5 I undertook the problems of *in vitro* probiotic properties characterization of selected *Lactobacillus* strains. *In vitro* tests allow for the initial selection of candidate strains before proceeding to more complicated and costly *in vivo* tests. Well-planned and conducted *in vitro* studies have a large impact on the effects observed in living organisms.

In the case of probiotic studies, the problem is the lack of detailed guidelines or manuals in methodology. The guidelines collected in the FAO / WHO Working Group report (2002) for the evaluation of new probiotic strains include, but are not limited to, survival at gastric juice, resistance to bile salts, mucus and / or human epithelial cells adhesion, antimicrobial activity against bacteria potentially pathogenic, the ability to reduce the adhesion of pathogens to the epithelial surface, the activity of bile salts hydrolases. In publications H1, H2 and H3 I proposed *in vitro* test in accordance with FAO / WHO guidelines (2002) and presented the results of research on *Lactobacillus* strains isolated from pickled cucumbers and cabbage in comparison to strains with documented probiotic properties.

In the studies included in the H1 publication, ten pre-selected *Lactobacillus* strains were evaluated for resistance to conditions simulating the human gastro-intestinal tract. It was considered that the most difficult barriers to overcome by bacteria during passage is the low pH prevailing in the stomach and the presence of bile salts in the small intestine. Two experiments were carried out as part of the research. In the first, cultures of the tested *Lactobacillus* strains were subjected to a 2-hour incubation in an environment of pH 1.5; 2.5 and 3.5 at 37 ° C to simulate the conditions in the stomach during digestion. The pH value 1.5 corresponds to the situation in the human stomach, when there is an accumulation of large amounts of gastric juices, while the pH value 3.5 represents the transitional environment between the stomach and the duodenum (Traczyk, 1989). **As a result of the conducted research, I found that in conditions of pH 3.5 and 2.5 the survival of *Lactobacillus* strains was above 90%, while the pH value of 1.5 caused a significant decrease in the number of living bacteria cells after 60 minutes of incubation to 23-38% and after 120 minutes no growth of microorganisms was observed.**

In the second experiment, *Lactobacillus* cultures were incubated on MRS medium supplemented with 0.2%, 2% and 4% bile salts at 37 °C. The physiological concentration of bile salts, involved in the digestive process in the small intestine is 0.3-0.6%. The concentration of bile salts at the level of 2% corresponds to the situation when there is a significant amount of food in the intestine. It is believed that bacterial resistance to high concentrations of bile salts as 2 and 4% correspond to their strong probiotic properties (Kurman, 1988). **As a result of the experiment, I found that all tested *Lactobacillus* strains tolerated 0.2% concentration of bile salts, while 2 and 4% concentration of bile salts was a discriminatory factor, that showed *Lb. brevis* O22 and *Lb. plantarum* K1 being resistant to these conditions.** Bacterial resistance to high concentrations of bile salts may result from the activity of the bile salts hydrolase (BSH) enzyme, produced as a protective factor against toxic effects of bile salts on LAB cells. BSH catalyzes the hydrolysis of taurine-conjugated salts of bile to free bile acids and amino acids, reducing their emulsifying capacity (Ziarno, 2005). The BSH activity of the lactic acid bacteria is not only a strain dependent, but also related to the origin of the strain - it is most often detected among intestinal strains, as confirmed by our own studies (H1).

In addition, the tolerance of the tested *Lactobacillus* strains to a 0.4% concentration of phenol in the environment was determined. Phenols can be formed in the intestine as a result of bacterial deamination of certain aromatic amino acids and are considered potential carcinogens (Suskovic et al., 1997). Some LAB strains are tolerant to phenol. These properties are desirable.

Based on the research (H1) I found that the *Lactobacillus* strains isolated from fermented products characterized good, comparable to the reference strains, with documented probiotic features, survivability under the model conditions described above. The *Lb. johnsonii* K4 and *Lb. brevis* O22 strains were characterized by a particularly best resistance to model conditions. The obtained results suggest that the tested *Lactobacillus* strains will be able to survive the gastro-intestinal passage and reach the colon, maintaining its viability. However, to confirm this thesis, *in vivo* human studies should be carried out.

This proves the rightness of the thesis, which suggests that bacteria contained in food are a source of commensal bacteria or at least temporarily present in the human gastrointestinal tract. Isolation of potential probiotics from the gastrointestinal tract is therefore not a just right idea. The assumption that bacteria isolated from the large intestine or from faeces survive digestion is obvious, however, as our own study presented in H1 and other research presented in H5 shows, lactic acid bacteria isolated from unconventional sources are also able to survive in such difficult conditions. Especially bacilli of the *Lactobacillus* genus, which tolerate the acidic environment well and are able to regulate the intracellular pH, have the above-mentioned properties.

In vitro tests provide important information on differences between species and strains and are an important research tool, especially for rapid screening towards probiotic activity. Research methods using static *in vitro* models used in work (H1) do not provide comprehensive information on the state of bacteria during digestion of food. Many factors influence the effectiveness of probiotic bacteria survival, including initial concentration of bacteria at the time of administration, the method of administration (the protective role of food ingredients), bacterial activity and their properties. To determine the impact of these factors on the survival of probiotic bacteria during *in vitro* digestion, an innovative tool - dynamic gastric model (DGM) was used, and the results of experiments were presented in the paper H2.

DGM was developed at the Institute of Food Research (Norwich, UK) and is a computer-aided *in vitro* model that simulates both biochemical and mechanical aspects of digestion in the stomach in real time (Thuenemann et al., 2015). I used the DGM model when I was at scientific internship in Denmark, at the University of Copenhagen, where I conducted experiments to determine the impact of the matrix (milk chocolate and 72% dark chocolate) on the survival of probiotic bacteria *Lactobacillus acidophilus* NCFM® and *Bifidobacterium animalis* subsp. *lactis* HN019 (HOWARUTM, Danisco USA Inc., USA). The digestion of probiotic chocolate in the DGM model showed that the population of *B. lactis* HN019 did not change significantly, regardless of the matrix used,

while the number of *Lb. acidophilus* NCFM® in both chocolate samples decreased significantly, most likely due to the negative impact of the model environment, where the pH value was less than 3.0 at the end of the digestion.

Comparing the results of the experiment with the use of DGM with the classic static model (H2), similar trends in bacterial survival were found. The influence of the concentration of applied bacteria on their survival during digestion in the static model was noticed. It was found that a dose of 10^8 CFU / g is insufficient to ensure an adequate level of bacterial survival during the digestive process. Bacterial cells have buffering capacity of environment, which in a high concentration may increase their chances of survival (Mortensen et al., 2006).

In order to compare the effect of other food matrices on the probiotic bacteria survival, digestion in the DGM model was made including selected commercially available probiotic products: Actimel ™ yoghurt drink, Gut & Günstig yogurt and Pro Viva - fruit juice and compared to the tested chocolates (H2). **It was noted that the survival of probiotic bacteria during 65 minutes of digestion is varied and depends on the food carrier. The best medium was chocolate, which contains proportionally more fat, compared to the other test carriers, which protects bacterial cells against digestive factors. Despite the fact that the content of fat in dark chocolate is 10% higher than in milk chocolate, the survival of probiotic bacteria was slightly higher in the case of milk chocolate. This may indicate a synergistic effect of fat and protein, the content of which was in turn higher in the case of milk chocolate**.

It seems that the high buffering capacity of the milk chocolate carrier may protect the probiotic bacteria from stress associated with the low pH prevailing in the stomach (Lahtinen et al, 2007). For this food medium, the bacterial count was maintained at a high level of> 10^9 CFU / g, whereas in dairy products the population of probiotic bacteria after 10-15 minutes of *in vitro* digestion decreased to the limit of detection and remained below this level until the end of the process digestion. Population of *Lb. plantarum* 299v - a probiotic strain that was a component of fruit juice, also decreased significantly (by about 4 logarithmic units) during the first 5 minutes of digestion and remained low (around 10^3 CFU/ g) until the end of the digestive process.

As a result of the research presented in the publication H2, I verified positively the hypothesis 2, demonstrating that selected strains of *Lactobacillus* bacteria isolated from spontaneously pickled vegetables can survive in difficult model conditions prevailing in the stomach and intestine, suggesting that they can reach the colon. I also proved that the food matrix is an important factor conditioning the survival of probiotic bacteria during digestion under *in vitro* models of human digestive tract, and the initial concentration of bacteria in food affects their survival during the digestive process. Thus, the selection of a suitable food vehicle and a suitable dose of probiotic bacteria is crucial in the design of functional foods process, because it determines the survival of the bacteria during digestion and thus the possibility of inducing a therapeutic effect.

Analysis and evaluation in vitro of selected probiotic properties chosen Lactobacillus strains

In the next work (H3), 10 strains of Lactobacillus isolated from fermented food and selected as a result of the research presented in the H1 work, meeting the elementary criteria included in the FAO / WHO recommendations (2002), were evaluated regarding the adhesion capacity to enterocyte Caco-2 line, the ability to regulate the production of cytokines by macrophages; and the effect of direct action of *Lactobacillus* cells on enterocyte apoptosis.

It was found that the cell adhesion of the tested *Lactobacillus* strains to enterocytes of the Caco-2 cell line is comparable to the adhesion capacity of the probiotic strains (*Lb. plantarum* 299v and *Lb.rhamnosus* GG), isolated from the human gastrointestinal tract (H3). Moreover, six of the tested strains (O12, O16, O18, O22, O24 and K3) showed greater adhesion capacity than the reference strains. The ability of *Lactobacillus* to adhere could be explained by the hydrophobic properties of these cells, which are considered an indirect measure of the ability of bacteria to adhere. The hydrophobic surface of bacterial cell walls allows for increased interaction with the mucus surface secreted by enterocytes. In our own research (H1), the hydrophobicity of BATH was found to be varied (24.7% - 73.4%) expressed as a degree of affinity for xylene. However, no correlation was found between the hydrophobicity of bacterial cell walls and the ability to enterocytes (H3) adhesion. It seems that the ability of the bacteria to adhere to the surface corresponds to many factors, among which the hydrophobicity of the cell walls is not a key factor. The factors that affect the ability of *Lactobacillus* bacteria cells to adhere, beyond the environmental factors, are protein factors, called adhesins, and non-protein factors such as lipotichoic acids and exopolysaccharides (EPS). It is suggested that EPS can also affect the ability to form aggregates and biofilms. These factors (both protein and non-protein) are closely related to the cell wall surface (Paliwoda and Nowak, 2017).

In further research I plan to develop the topic of the adhesion ability of *Lactobacillus* cells. In the project Miniatura 1 (No. 2017/01 / X / NZ9 / 01627), of which I was the head investigator, I investigated the influence of thermal damage of *Lactobacillus* cells on the adhesion capacity. The aim of the study was quantitative - qualitative comparative assessment of the ability of *Lactobacillus* cells to adhere and aggregate depending on their form (live cells and dead), as well as initial identification of substances responsible for the expected effect. The results of these experiments are promising and suggest that damaged bacterial cells also have the ability to adhere and bind intestinal mucus.

It is believed that the selection of an appropriate probiotic strain for use in food or in probiotic supplements should be based on its ability to improve the immune response in the gut (Galdeano et al., 2007, Dong et al., 2010). In the H3 publication, the effects of the addition of *Lactobacillus* strains on the regulation of the *in vitro* secretion of selected cytokines (IL-1β, IL-6, IL-10, IL-23 and TNF-α) by macrophages were investigated. **The**

results of the experiments suggest that the tested *Lactobacillus* strains do not stimulate the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-23 and TNF-α) by LPS-induced macrophages (lipopolysaccharide derived from the *E. coli* cell wall), and even they can inhibit their secretion (e.g., IL-6 and IL-23).

It should be noted that IL-6 has both pro- and anti-inflammatory properties. It plays an important role in identifying the initial inflammatory reaction. The presence of IL-6 may help to transform the innate response to a more specific and sustained adaptive response to pathogens (Gabay, 2006). The immune response of macrophages induced only by tested *Lactobacillus* strains was about 100-fold lower compared to LPS stimulation, which indicates a low level of stimulation of the inflammatory response by *Lactobacillus* bacteria themselves. **Moreover, most of the tested strains stimulated the production of anti-inflammatory IL-10 cytokine, with the strongest stimulator strains were *Lb. plantarum* O20, as well as *Lb. brevis* O22 and O24 (H3), this phenomenon was not dependent on LPS stimulation. The presented research results show a high potential of the tested *Lactobacillus* strains for immunomodulation.**

Subsequent experiment (H3) consisted in the evaluation of the effect of live cells of the tested *Lactobacillus* strains on apoptosis of enterocytes. Apoptosis is the planned death of the cell, thanks to which the number of cells in tissues is controlled, and single, e.g. mutant cells are eliminated. However, unplanned apoptosis of some cells may be harmful (Howarth and Wang, 2013). In study (H3), it was shown that *Lactobacillus*-induced apoptosis was accompanied by changes in caspase-3 activity, which is one of the first signals to start in the cell of this process. It has been shown that *Lactobacillus* cells may slightly induce apoptosis in Caco-2 cells. The strain with the strongest properties of apoptosis stimulation was *Lb. casei* O18. On the other hand, the studied *Lactobacillus* strains did not increase chemically induced apoptosis (by staurosporin - STS), and seven of them (*Lb. casei* O16 and O18, *Lb. plantarum* O19, O20, *Lb. brevis* O22, *Lb. rhamnosus* K3i *Lb. johnsonii* K4) decreased the activity of caspase-3 after STS stimulation, but these changes were not statistically significant. In conclusion, it was shown that the tested *Lactobacillus* strains have the ability to induce apoptosis, but at the same time they do not increase chemically induced apoptosis, which may have consequences and application in the regulation of vital cell processes. Undoubtedly, a better understanding of intestinal interactions can accelerate the development of new therapeutic strains for human use.

In my own research presented in H1 and H3 I showed that due to the resistance to the *in vitro* conditions in the gastrointestinal tract and adhesion capacity, the tested *Lactobacillus* strains isolated from traditionally fermented food will most likely be able to colonize the intestinal epithelium and induce immunomodulation, which positively verifies the hypothesis 3.

Due to the fact that the experiments carried out in the works H1, H2 and H3 were *in vitro* studies, the conclusions from them are conditional, because not always *in vitro* tests are compatible with the *in vivo* effect in the human body. Undoubtedly, the weakness of most of the researches describing the probiotic properties of strains isolated

from various unconventional sources are evidence collected only on the basis of *in vitro* studies, which was noted in the publication H5. Based on a comparison of 60 original research papers from recent years, it was found that only a few studies were conducted on experimental animals, and even fewer experiments were conducted with human participation. It should be emphasized that *in vivo* studies are more costly and time-consuming, therefore, the more important role is played by the initial effective selection of candidate strains for further research towards the definition of the probiotic effect (H5).

In the works H1, H2 and H3 I proposed a set and sequence of a series of *in vitro* tests of new bacterial isolates with high discriminative power, and at the same time simple and cheap. Such tests may allow for initial, rapid selection of strains, prior *to in vivo* testing, which has both scientific and application value.

Analysis and evaluation in vitro of selected probiotic properties chosen Lactobacillus strains

An important advantage of LAB strains isolated from food is their ability to grow and take up metabolic activity in these products. Technological properties that are desirable from the producer's and consumer's point of view are: the ease of production of large amounts of biomass, high production productivity, resistance to bacteriophages, genetic stability, resistance to primer fixation procedures (freezing, lyophilization, storage), viability and stability of desired bacterial traits during the preparation and distribution of probiotic products, high storage stability of bacteria in the finished product as well as ensuring the desired sensory characteristics of finished products (Nowak et al., 2010).

Goat milk has valuable nutritional values, but at the same time it is a difficult raw material, due to its unattractive and, for some, unacceptable sensory characteristics, which largely determine consumer choices. The solution that increases the attractiveness of this product can be fermentation. It was noted that fermented beverages from goat's milk are characterized by high biological value and protein digestibility as well as satisfactory sensory characteristics (Garcìa et al., 2014). Following this trend in food technology, it was decided to use probiotic and potentially probiotic bacteria for the fermentation of this raw material, in order to add a new functional value (pro-health).

The research presented in the H4 publication attempts to use the potentially probiotic *Lb. rhamnosus* K3 and *Lb. plantarum* O20 strains selected in earlier studies H1 and H3, for the production of fermented beverages from goat's milk. For comparison, two probiotic, commercially available strains *Lb. acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12, from the collection of Chr. Hansen were used. Tested strains were used as starter monocultures, conducting the process of fermentation of milk by tank method at 37 °C until acidification (pH 4.5-4.8). Beverages prepared in this way were evaluated for microbiological and sensory quality during 0, 3, 7, 10 and 14 days of storage at 4 °C.

As a result of the tests (H4) it was found that the initial number of cells of introduced bacterial strains was high (above 10^8 CFU / ml) and remained at this level throughout the storage period. For the most desirable according to the taste criterion, the evaluators considered the goat milk fermented with the *Lb. plantarum* O20 strain. Drink fermented with the *Lb. plantarum* O20 was characterized by the highest notes of general quality, as well as smell and milk-fermentation flavor and smoothness. In addition, fermentation with addition of this strain masked the "goat" taste and smell - typical for goat's milk. Fermentation of goat milk with the *Lb. rhamnosus* K3 was characterized by average notes of general quality, which were influenced by high notes of sour taste and not very high notes of smoothness. The fermented drink with the *Lb. acidophilus* La-5 strain was rated the worst, which was also characterized by the highest intensity of notes of sour taste, as well as quite intense bitter, irritating and "goat" flavor. In turn, the drink fermented with the *Bifidobacterium animalis* BB12 strain was characterized by a mild flavor and aroma, unusual for dairy fermented beverages. Significantly higher intensity of the sweet taste and lower acidic and milky fermentation taste, compared to the other variants of beverage samples, made the desirability of this drink moderate.

Based on research presented in the H4 publication, it can be concluded that the use of different strains of probiotic and potentially probiotic bacteria for the fermentation of goat's milk determines highly the sensory quality of final product. The technological usefulness of the bacteria used in the study was varied, and the best sensory quality of beverages were made with the *Lb plantarum* O20 strain, which was isolated from pickled cucumbers.

Thus, I proved that it is possible to produce functional fermented beverages from goat's milk, with a sufficiently high dose of living bacteria cells in 1 g of the product, which allows the food to be considered as functional. I found that the technological suitability of the strains varied and depended on the sensory quality of the obtained beverages, positively verifying the hypothesis 4.

Strains of *Lactobacillus* bacteria isolated and characterized by me in the presented publications H1, H3 and H4 have also found application in the works of other authors (Sionek et al., 2015, Sionek et al., 2016, Trząskowski et al., 2018) and own work (Szydłowska et al. ., 2017). It has been proven that selected strains of *Lactobacillus* bacteria isolated from fermented foods in a traditional way may find application in the design of functional foods (vegetable juices, honey drinks, pumpkin sorbets). New strains of bacteria with potentially probiotic features are deposited in the internal collection of the Chair of Food Hygiene and Quality Management of the Warsaw University of Life Sciences.

Summary

The series of publications presented as an achievement is a multi-stage study dealing with the problem of acquiring probiotic strains for human use. In the presented

achievement, I characterized functional features and assessed the technological suitability of *Lactobacillus* strains, which I isolated from traditional fermented food.

As a result of my own research, and based on the analysis of research papers published in the subject literature in recent years, **I proved that food is a good source of isolation of bacteria with probiotic features. Moreover, selected bacterial strains are characterized by better suitability for functional food development, with attractive sensory properties, compared to human gastrointestinal tract isolates,** which I consider to be the most important achievement in my current scientific and research work. An additional achievement **is the proposition of a comprehensive set of *in vitro* tests to select candidate strains for further *in vivo* studies. The proposed sequence of rapid *in vitro* tests allows for the selection of safe bacteria strains, resistant to low pH environment and high concentration of bile salts, as well as showing the adhesion ability to enterocytes and immunoregulatory potential.**

The obtained research results provide evidence and knowledge about the probiotic activity of *Lactobacillus* bacteria isolated from food, which is connected with their pro-health activity in the human body and predisposes for further research, towards defining the probiotic effect *in vivo*. The research presented in my achievement has also of significant application character. The tested *Lactobacillus* bacteria can be used as starter cultures for the production of functional foods.

I consider the following as most valuable achievement resulting from the research presented for evaluation:

- Demonstration that traditional fermented food is a good source of isolation of bacteria with properties defined as probiotic, which are comparable to strains with documented probiotic properties, isolated from human digestive tract.
- Optimizing and proposing a set and sequence of *in vitro* research methods that aim to provide fast and adequate discriminatory power tests for screening bacteria for their probiotic features.
- Demonstrating that the selection of a suitable food vehicle and the appropriate dose of probiotic bacteria is crucial in the development of functional foods, because it determines the survival of the bacteria during digestion *in vitro* and, consequently, the possibility of inducing a therapeutic effect.
- Demonstrating that selected strains of *Lactobacillus* bacteria isolated from food are safe for human use and can survive under conditions that simulate the gastrointestinal tract, exhibit the adhesion to intestinal epithelial cell *in vitro* and immunoregulatory activity.
- Demonstrating that selected strains of *Lactobacillus* bacteria isolated from food can be used to development of food with functional properties, due to better technological suitability, understood as obtaining a product with the desired sensory characteristics, compared to strains with documented probiotic features.

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5. Summary of other scientific and research achivements

Before doctoral degree

I did my research for the master's thesis in 2002 at the Department of Gastronomic Technology and Food Hygiene under the scientific supervision of Małgorzata Jałosińska, Ph.D. In these studies I conducted the research on assessment of the survival of probiotic bacteria in a developed carrot drink. Positive results of research, as well as the desire to explore knowledge about the use of probiotics in food led me to take up doctoral studies, which I was started in 2003. I conducted research for the doctoral dissertation in the same Chair under the scientific supervision of prof. dr hab. Danuta Kołożyn-Krajewska, later promoter of my doctoral dissertation. The aim of the work was to design a soy beverage fermented with a probiotic bacterial strain and to construct mathematical models predicting the survival of these bacteria in a product under different conditions. As a result of the research, a probiotic soy drink was designed with acceptable sensory characteristics and a appropriate number of bacteria added (*Annex 4 II.1 and II.D.2* and *III.B.1, III.B.2*). The appropriate conditions for maturation and storage of the drink were then selected and the survival rate of the bacteria in the designed beverage was assessed (*Annex IV II.3* and *III.B.3, III.B.4, III.B.5*). The next stage of the study consisted in the use of experimental data to construct predictive growth and survival models of probiotic bacteria in a soy beverage (*Zal.4 II.D.4 and III.B.6*). On December 17, 2008, I defended my doctoral thesis entitled: "Predictive models of survival of probiotic bacteria in fermented soy beverage" at the meeting of the Council of the Faculty of Human Nutrition and Consumer Science SGGW in Warsaw, where I received a Ph.D. in agricultural sciences in the discipline food and nutrition technology, and my doctoral dissertation was honored.

The result of the work undertaken during the Ph.D. thesis were publications (*Annex 4 II.A.3, II.D.9, II.D.15*), conference report (*Annex 4 III.B.7*), patent application (*Annex 4 II.B.1*), as well as the implementation, consisting in placing the models constructed in the Prognostic Database (*Annex 4 III.A.2*), which is made available free of charge for training and industrial use (http://prognostycznabazadanych.sggw.com /). These effects have been published after the doctoral thesis, which will be discussed below.

After doctoral degree

In December 2008, I was employed as an assistant at the Department of Food Technology and Food Hygiene at the Faculty of Human Nutrition Sciences and SGGW in Warsaw, and then from 2010 until today I work as an adjunct. After defending my doctoral thesis, I undertook scientific and research activity in many areas, among which the following thematic groups can be distinguished:

- Isolation, identification and genotyping of lactic acid bacteria;
- Safety of using lactic acid bacteria, their antimicrobial properties and resistance to conditions simulating the gastrointestinal tract;
- Technological use of probiotic bacteria;
- Mathematical modeling and microbiological quality of food.

5.1. Isolation, identification and genotyping of lactic acid bacteria

In the years 2011-2013 I took part in a research project of the Executive Program with the Institute of Microbiology of the Belarusian Academy of Sciences, Republic of Belarus on: "Attempt of isolation and characterization of probiotic bacteria and use in selected food products" as the main investigator on the part of SGGW. As part of this project, I undertook the first attempts to isolate and pre-identify the phenotypic bacteria of lactic fermentation from food. The research resulted in osters on conferences (*Annex 4, III.10 and III.13*), and also prompted me to continue work on the isolation, identification and characterization of bacteria. The effect of this project was also the use of probiotic bacteria in the development of functional foods, which will be discussed in Section 5.3.

Next, I obtained funding as a research task manager within the internal competition mode at the Faculty of Human Nutrition and and Consumer Sciences, SGGW in Warsaw, for a young scientist in three subsequent years: in 2012: "In vitro studies of selected properties of probiotic lactic acid bacteria isolated from traditional fermented food ", in 2013 on:" *Biotechnological usefulness of lactic acid bacteria with potentially probiotic properties (continuation of research)* "and in 2014 on" *Genotyping of lactic acid bacteria isolated from food*".

In 2013, I also completed a scientific internship at the Department of Plant Genetics, Breeding and Biotechnology, SGGW under the scientific supervision of prof. dr hab. Z. Przybecki, where by participating in the project "*Methodological fundamentals of transformation of cucumber (Cucumis sativus L.) high molecular weight DNA*" I gained valuable knowledge and skills in the field of genetic and molecular biology methods. The result of this cooperation was also a publication about new molecular biology methods and next generation sequencing techniques, in the preparation of which I had the honor to participate (*Annex 4 II.A.4*). Thanks to the experience gained in the laboratory of molecular biology, I was able to organize my own work workshop and start work on the genotyping of lactic acid bacteria that I isolated from food.

The result of the implementation of projects as well as own research works is a large collection of new bacterial strains, among which 78 have been identified so far, and their nucleotide sequences are published in the GenBank NCBI database (*Annex 4 II.E.1-4*). The source of isolation of lactic acid bacteria strains was food produced in a traditional way, ie: ecological raw cured meat (*Annex 4 II.A.7, II.D.18* and *III.B.26*), regional cheeses oscypek and koryciński (*Annex. 4 II.A.5*), organic acid whey (*Annex 4 II.A.6*) and pickled cucumbers and cabbage, which were discussed above (*Annex 4 IB1*). The collection of new strains deposited in the Department of Food Hygiene and Quality Management of the Faculty of Human Nutrition and Consumer Sciences is presented in the publication in the industry magazine Przemysł Spożywczy (*Annex 4 II.D.12*). These strains can be used as starter cultures or protective cultures for fermented food production.

The experience that I gained while undertaking the issue of bacterial identification at the strain level I used in publication (*Annex IV II.13*) and presented at the conference (*Annex 4, III.B.9*). This publication focuses on the problem of isolating bacteria from food and identifying methods that could be helpful in industry or inspection to confirm the presence of probiotic bacteria in food at the strain level. This is crucial because the probiotic properties are strain-dependent. In another publication (*Annex 4 II.D.22*) special attention was paid to the problem of bacterial culture. According to the current state of knowledge in food products, especially those with low pH values and stored at low temperatures, bacteria can be live but not able to growth on laboratory media, called VBNC (viable but non culturable state). In this case, classical methods, based on growing the bacteria on the media, become unusable. This study analyzed the usefulness of microbiological, biochemical, genetic, proteomics and other molecular techniques. It was found that, according to the current state of knowledge, the best method that could help in a reliable determination of the probiotic bacterial population at the strain level is flow cystometry, as well as a combination of culture and PFGE electrophoresis methods.

5.2. Safety of using lactic acid bacteria, their antimicrobial properties and resistance to conditions simulating the gastrointestinal tract

From 2013, I was the auxiliary promoter of Anna Rzepkowska's doctoral dissertation (currently Anna Łepecka, Ph.D.), and previously a supervisor of her master's thesis. The title of the doctoral thesis was: "*In vitro evaluation of the probiotic properties of lactic acid bacterial strains isolated from food*". The scope of work included the isolation of lactic acid bacteria from raw cured meats and acid whey, as well as their assessment of safety and survival in conditions simulating the gastrointestinal tract. My role encompassed scientific care, assistance in organizing the workshop and in the optimization of research methods, as well as supervision over the implementation of experimental works. This work was completed and the defense of the doctoral thesis took place in October 2017.

In parallel from 2014, I am the auxiliary supervisor of Aleksandra Ołdak's doctoral dissertation, and earlier I was a supervisor of her master's and engineer's thesis. The

subject of the doctoral dissertation is: "*Evaluation of antimicrobial properties of lactic acid bacterial strains isolated from food*". The scope of work includes a comparative assessment of antimicrobial properties *in virto* of bacteria strains isolated from regional cheeses: oscypek and koryciński. My role is to provide scientific care, help in the organization of workshop and in the optimization of research methods, as well as supervision over the implementation of experimental works. At present, the research work has been completed and the doctoral student submits doctoral examinations.

Thanks to close cooperation with doctoral students, we managed to construct a unique research team, organize a workshop and conduct a series of experiments and analyzes. This cooperation has resulted in numerous publications and reports on conferences, which will be discussed below.

According to FAO / WHO recommendations (2002) during the assessment of the probiotic properties of bacteria, the next step, after proper identification, is the safety assessment. In my research I also dealt with this subject. The publication (*Annex 4 II.10*) discusses the criteria that are taken into consideration in the *in vitro* safety assessment. Particular attention has been paid to the antibiotic resistance profile, thanks to which pre-selection of candidate strains is possible. Antibiotic resistance is not only a problem occurring among pathogenic bacteria, more and more reports also indicate that *Lactobacillus* bacteria may be a specific reservoir of antibiotic resistance genes. In the studies presented in the publications (*Annex 4 II.A.6* and *7* and *Annex 4 I.B.1*), the antibiotic resistance profile of strains of lactic acid bacteria isolated by me was determined using E-tests, i.e. strips impregnated with a gradient of specific antibiotics. In addition, strains isolated from raw-cured meat were genotyped and the presence of genes coding resistance to known antibiotics in bacterial genomic DNA was confirmed. In the same studies (*Annex 4 II.A.6* and *7* and *Annex 4 I.B.1*), the antibiotic resistance profile was supplemented with the analysis of the enzymatic activity of the tested bacterial strains, thanks to which it was possible to select and exclude potentially dangerous strains. The results of the research were also presented at conferences (*Annex 4, III.B.18, III.B.23, III.B.28*).

However, the assessment of the safety of using probiotic bacteria in humans can not be based only on *in vitro* tests. An important element is research conducted on laboratory animals (espetially immunodefective) and clinical trials on large population groups. It is believed that probiotics may be theoretically responsible for four types of adverse effects: systemic infections, harmful metabolic activity, excessive stimulation of the immune system in susceptible individuals and gene transfer. A detailed analysis of these potential adverse reactions was carried out at work (*Annex 4 II.D.21*).

The publication (*Annex 4 II.A.8*) presents the problem of increasing antibiotic resistance, which also applies to LAB strains used as starter cultures for food. It was noted that the mechanisms of action of antibiotics and bacteriocins (low-molecular proteins, produced inter alia by lactic acid bacteria) are similar. The key difference between these compounds is the fact that microorganisms do not generate resistance to

bacteriocins, unlike antibiotics. An undoubted advantage of bacteriocins is also their selective mode of action, which can contribute to the protection of biodiversity of the microbiota of the human digestive tract. The problem that limits the use of bacteriocins is to determine the effective route of administration to patients.

An important element in the assessment of probiotic bacteria is their antimicrobial properties, which was presented in the paper (*Annex 4 II.D.19*). In turn, in publications (*Annex 4 II.A.5, II.A.6* and *II.A.7*) and reports on conferences (*Annex 4 III.B.22, III.B.25, III.B. 27, III.B.31, III.B.35, III.B.38, III.B.42*), evaluation of the antimicrobial properties *in vitro* was carried out, among others using the well diffusion method, or evaluation of inhibition of adhesion of indicator microorganisms to the Caco-2 cell line. It has been shown that lactic acid bacteria isolated from food have a strong activity against *Listeria monocytogenes,* as well as different effects against *Salmonella, Escherichia coli* and other spoilage microorganisms. Reports on conferences also noted the antagonistic properties of *Staphylococcus aureus* (*Annex 4, III.B.39*). In many cases, the antibacterial activity was maintained after removal of live lactic acid bacteria from the medium as well as after neutralization of the obtained supernatant. This suggests that some of the tested bacterial strains may produce bacteriocins or other low molecular weight proteins with antimicrobial properties. Moreover, it has been noted that these properties depend on the source of insulation.

Resistance to conditions in the human digestive tract, understood as the low sensitivity of bacteria to enzymes, bile salts and low pH is one of the key criteria for probiotic strains. For the health effect to occur, the bacteria must reach the large intestine. Because I found this problem extremely important, I decided to develop this subject in my scientific work. In searching for a research methodology, I came across a multitude of solutions. In my work (*Annex 4, II.D.24* and *Annex 4, IB1*), I used simple tests of bacterial survival under given conditions, while at work (*Annex IV II.6*) I constructed (together with a Ph.D. student Anna Rzepkowska) a static *in vitro* model, which takes into account the continuity of the digestive process. In turn during my stay at a scientific internship in Denmark (*Annex 4 I.B.2)* I determined the survival rate of bacteria in a dynamic model, which was discussed in the above. Selected research results were also presented at conferences (*Annex 4, III.B.14, III.B.19, III.B.34*). As a result of these experiments, I noticed that a key discriminant factor among those used in experiments is the low pH of gastric juice. Most of the bacteria tested were sensitive to pH 1.5, but a certain proportion of the bacterial population, depending on the strain, was able to survive at pH 2.0, while pH 3.5 does not significantly affect the number of living bacteria cells during digestion. Importantly, it was noticed that the addition of milk (*Annex 4 II.D.6*) and cream (*Annex 4 III.B.40*) protects the bacterial cells and increases their survival in the *in vitro* gastrointestinal tract.

In summary, it was noted that some bacteria isolated from food may have properties defined as probiotic. In the publication (*Annex 4 II.D.23*), the legal aspects related to the presence of probiotic bacteria on the food market were discussed in detail, as well as their origin. While using the results of own research and other authors, it was found that it is

crucial to prove the possibility of bacterial survival under the conditions of the human digestive tract, regardless of the source of isolation, in order to expect a probiotic effect. The results of our own research related to the topic of *in vitro* probiotic properties were also presented at conferences (*Annex III B.20, III.B.24, III.B.37*) and a patent application for a new strain of *Lactobacillus brevis* and its application was prepared (*Annex 4 III.B.2*).

5.3.Technological use of probiotic bacteria

My research interests are also focused on the use of probiotic and potentially probiotic bacteria in food product development. Probiotic bacteria are most often a component of fermented milk beverages as well as other dairy products. The research direction that I have developed in my work is the attempt to use other raw materials (plant and animal origin) as carriers of probiotic bacteria. The first raw material that I used in my research was soy. Possibilities of using bacteria with probiotic features for the production of fermented soybean drink are presented in the works (*Annex 4 II.A.2, II.D.9, II.D.15*), and in the work (*Annex IV II.7*) to tofu production. Other carriers of probiotic bacteria that were analyzed were carrot juice (*Annex 4 II.D.16*) and pumpkin and apple mousse (*Annex 4 II.D.8*). The results of these studies were also presented at numerous conferences (*Annex 4, III.B.8, III.B.11, III.B.12, III.B.13, III.B.16, III.B.41*).

Raw materials of animal origin were also tested as carriers of probiotic bacteria. A probiotic milky fermented beverage with a pineapple-coconut flavor was designed (*Annex III, III.2B*), synbiotic fermented milk (*Annex III, III.B.29*), a potentially probiotic milk drink (*Annex III, III.B.30*), probiotic, fermented beverages from goat's milk (*Annex 4 III.B.36 and IA4*), as well as a probiotic milk-chocolate product (*Annex 4 III.B.33*).

Taking part in scientific projects: "*Ecological methods of meat processing and production of meat products without the use of nitrite and nitrite additives, including the extension of shelf life of these products*" in 2013, as well as "*Processing of plant and animal products using ecological methods. Research on innovative solutions in the field of meat processing, with the reduction of nitrite and nitrite additions and simultaneous extension of storage life*" in 2017. and "*Research on innovative solutions in the field of meat processing, with the reduction of nitrite and nitrite additives, including the use of fermented milk of various animal breeds in the processing of meat in order to affect health, sensory parameters and stability of products*" in 2018, the purpose was to use probiotic bacteria for the production of raw cured meats, I noticed that meat is a good carrier for probiotic bacteria. Possibilities of using bacteria with probiotic properties in the industrial production of maturing sausages are presented in the papers (*Annex 4 III.B.32, III.B.43*). In addition, it was noted that starter cultures in the form of probiotic bacteria can increase food health safety, through the production of bacteriocins (*Annex IV II.D.14*), whether to influence the quality by shaping physico-chemical and sensory characteristics (*Annex IV II.D.20*) of raw fermented meat products. The risk analysis of the use of probiotic bacteria in meat products is presented in the works (*Annex 4 II.A.1* and *II.D.5*).

5.4.Mathematical modeling and microbiological quality of food

The effect of the research undertaken during the doctoral thesis was the construction of mathematical models that can be used to predict the microbiological growth and survival of probiotic bacteria in fermented soy beverage. These models were made available on the Internet platform (*Annex 4 III.A.2*), and published research (*Annex 4, II.A.3*). In the later course of scientific and research work, I did not continue my research into mathematical modeling.

The quality of probiotic products in the consumers' opinion was the subject of the work (*Annex 4 II.D.17*). In the reports on conferences I also indicated the problem of probiotic food quality (*Annex 4 III.B.15, III.B.17*). In these works special attention was paid to the analysis of the possibilities of satisfying the needs of consumers. It has been shown that the reason for not buying probiotics are: higher price, lack of faith in the action of such products, distrust of probiotic products and lack of satisfaction with the effects of action. Nevertheless, it was found that the majority of the population attaches importance to a healthy diet and is open to market novelties. These factors are important from the point of view of food producers.

Another area that I have undertaken in my work is the microbiological quality of food. The publication (*Annex 4 III.D.11*) discusses the problem of psychrotrophic bacteria in the refrigeration storage of food, paying attention to new microbiological threats and the possibilities of preventing them. In articles (*Annex 4 II.D.26* and *II.D.27*), the problem of hygiene (especially of personnel) and hygiene monitoring in meat establishments was also undertaken.

An important element of my scientific and research work were also expert opinions and custom-made studies. These works concerned opinion about product innovation (*Annex 4 III.M1-.2*), while custom-made works - assessment of the microbiological quality of newly designed products (*Annex 4, III.3, 3-5*).

In 2017 and 2018 I was an investigator in projects financed by MRiRW "*Processing of plant and animal products with ecological methods: Research on optimization and development of innovative processing solutions to increase the value of healthy organic products*" Subsidy of the Minister of Agriculture and Rural Development for 2017 and "*Processing of plant and animal products using ecological methods: Research on innovative solutions to improve the characteristics and sensory parameters of organic fruit and vegetable processing products, taking into account the preservation of nutrients of the received products*" Subsidy of the Minister of Agriculture and Rural Development for 2018. The result of research in both projects was development of recipes for high-protein organic bars, as well as a comprehensive assessment of the quality of these products (nutritional value, microbiological quality, physico-chemical, sensory and texture). As part of the study, the shelf-life date was also specified and the reports were published (*Annex 4, II.E.3, II.E.4*). In 2018, as part of the summary of research for organic farming, I also conducted training for organic food producers and advisers at the Ministry of Agriculture and Rural Development.

Currently, I participate in a scientific project: "*Developing a system for monitoring wasted food and an effective program to rationalize losses and reduce food waste*", PROM acronym, financed by NCBiR as part of the Gospietateg 1/385753 / 1NCBR / 2018 competition. The scope of work in which I participate as a investigator includes determining the microbiological and physico-chemical quality of different categories of food, which is kept longer than the minimum durability declared by the producer. The aim of the activities is to determine the actual date decisive for food safety.

In the future I would like to continue my scientific and research work related to probiotics and broadly understood quality of food.

6. Summary of scientific achievements

No.	Publication	Number of publication		IF*	IF(5-year)	Points MNiSW**	Total points
		before PhD	after PhD				
A. Publications in scientific journals with the Impact Factor (IF), and indexed in the *Journal Citation Reports* (JCR)							
1	Biomed Research International (2017)	-	1	2,583	2,931	25	25
2	Biomed Research International (2018)	-	1	2,583	2,931	25	25
3	Current Microbiology	-	1	1,519	1,563	15	15
4	Fleischwirtschaft	-	1	0,112	0,096	15	15
5	International Journal of Dairy Technology	-	1	1,225	1,245	20	20
6	International Journal of Food Properties	-	1	1,845	1,610	25	25
7	International Journal of Food Science & Technology	-	1	2,383	2,173	25	25
8	LWT-Food Science and Technology	-	1	2,329	2,929	35	35
9	Plant Science	-	1	3,437	4,148	35	35
10	Postępy Higieny i Medycyny Doświadczalnej	-	1	0,783	0,820	15	15
11	Probiotic and Antimicrobial Proteins	-	1	2,345	2,091	20	20
12	The Journal of Microbiology	-	1	1,439	1,514	20	20
13	Żywność. Nauka. Technologia. Jakość	-	1	0,190	0,295	15	15
RAZEM:		**-**	**13**	**22,773**	**24,346**	**290**	**290**
B. Publications in scientific journal without the Impact Factor, included in the B List of MNiSW' journal rank							
1	Acta Scientiarum Polonorum Technologia Alimentaria	-	1	-	-	15	15
2	Przemysł Fermentacyjny i Owocowo-Warzywny	-	1	-	-	6	6
3	Przemysł Spożywczy	-	3	-	-	12	36
4	Zeszyty Problemowe Postępu Nauk Rolniczych	-	1	-	-	9	9
5	Żywność. Nauka. Technologia. Jakość	-	2	-	-	13	26
6	Żywność. Nauka. Technologia. Jakość	4	-	-	-	8	32
RAZEM:		**4**	**8**	**-**	**-**		**124**

C. Original research works published in English in other international scientific journals							
1	Fleischwirtschaft International	-	1	-	-	4	4
2	Journal of Microbiology, Biotechnology and Food Sciences	-	1	-	-	4	4
RAZEM:		**-**	**2**	**-**	**-**		**8**
D. Chapters in scientific monographs							
1	Research works published in English as chapters in a monograph	-	4	-	-	5	20
2	Research works published in Polish as chapters in a monograph	-	4	-	-	4	16
RAZEM:		**-**	**8**	**-**	**-**		**36**
E. Chapters published in Polish in academic course books							
1	Rozdział	-	1	-	-	-	-
RAZEM:		**-**	**1**	**-**	**-**	**-**	**0**
F. Publications in conference materials							
1	In the form of abstracts, in English	1	8	-	-	-	-
2	In the form of abstracts, in Polish	5	27	-	-	-	-
RAZEM:		**6**	**35**	**-**	**-**	**-**	**0**
G. Scientific articles for the general public							
1	Polskie Mięso	-	2	-	-	-	-
RAZEM:		**-**	**2**	**-**	**-**	**-**	**0**
H. Patent application							
1	Patent application	1	1	-	-	-	-
RAZEM:		**1**	**1**	**-**	**-**	**-**	**0**
I. Nucleotide sequences reported and published in the NCB GenBank database							
1	Sequences application	**-**	**78**	-	-	-	-
RAZEM:		**-**	**78**	**-**	**-**	**-**	**0**
Razem wszystkie publikacje		**159**		**22,773**	**24,346**	**-**	**458**

* Impact Factor according the year of publication
**Number of points according to Ministry of Science and Higher Education (MNiSW) journal rank assigned to a publication based on:
1. Standardized journal rank from the MNiSW website (2007-2010)
2. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 20 December 2012
3. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 17 December 2013
4. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 31 December 2014 (with amendments from the 19 December 2015)
5. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 23 December 2015
6. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 9 December 2016
7. Announcement of the Minister of Science and Higher Education on the list of scientific journals of the 26 January 2017

Summary

- The sum of points for publications, according to Ministry of Science and Higher Education journal rank in accordance with the year of publication, is: **458** (**426** after doctoral degree),
- The total impact factor (IF) of scientific publications according to the Journal Citation Reports (JCR) list, according to the year of publication, is **22.773** (**22.773** after doctoral degree),
- The number of papers published in journals indexed by Journal Citation Reports (JCR) is **13** (in total **290** points, which is **63.3%** of the total number of points),
- The Hirsch index of published papers according to the Web of Science database (25/03/2019) is: **4**,
- The number of citations according to the Web of Science database (25/03/2019) is: **61** without self-citations **58**, according to the Scopus database: **78**, without self-citations **71**.