



A REVIEW

On a dissertation work for acquiring the scientific degree "Doctor of science" in Higher education field 5. Technical sciences, professional field 5.13. General engineering, scientific major "Technology of milk and dairy products"

Thesis author:

Associate professor Dr Tsend-Ayush Chuluunbat, performing doctoral studies at the Department Animal Sciences at Agricultural University, Plovdiv.

Thesis topic: THEORETICAL AND EXPERIMENTAL SUBSTANTIATION OF THE FUNCTIONAL NUTRITION DAIRY PRODUCTS TECHNOLOGY IN THE CONDITIONS OF MONGOLIA

Thesis Reviewer: Prof. Dr. Angel Ivanov Angelov, University of Food Technologies, designated as a member of the scientific jury by Order № RD-16-824/13.07.2022 by the Rector of AU.

1. Brief introduction of the candidate

Assoc. Prof. Dr. Tsend-Ayush Chuluunbat completed a Master's program in Dairy Technology at Moscow State University of Applied Biotechnology, Russian Federation in 1993. At that time, she was a lecturer at the Mongolian University of Science and Technology. In the period of 1994-1997, she developed and defended a doctoral thesis in the field of Technology of milk and milk products at the East Siberian Technological University, Ulan-Ude, Russian Federation. Since 2005, she has been appointed as an associate professor successively at the Faculty of Industrial Technology and at the Faculty of Food Technology and Biotechnology at the Mongolian University of Science and Technology, where she is currently working.

2. Current state of the topic

The development of functional foods and their introduction on the food market is related to two main problems facing modern society - health and balanced nutrition. This has resulted in a huge variety of foods on the market in the last twenty years. A similar trend is reported for the market of healthy products in Mongolia. Revealing the potential of lactic acid bacteria isolated from traditional dairy products opens up opportunities both for some theoretical contributions and for their application as starter cultures in a number of technologies for obtaining functional dairy products and cheese. In this connection, the current doctoral dissertation is dedicated to an up-to-date scientific problem. The conducted scientific studies, the obtained results and evidence are of particular importance for the development of new children's and dietary dairy products and goat and sheep milk cheeses for the Mongolian market.

3. Aim, objectives, hypotheses and research methodology.

The aim of the dissertation work is to conduct in-depth scientific research, on the basis of which to elaborate technologies for obtaining a variety of functional dairy products using developed starter cultures of lactic acid bacteria isolated from traditional Mongolian dairy products. The aim of the work is clearly and precisely formulated, and the more than ten main objectives are well defined, specific and aimed at achieving the aim of the study.

Various modern methods have been applied in the presented research work - chemical, microbiological, molecular genetic for the identification of lactic acid bacteria, selective methods based on assessment of the probiotic characteristics of the identified strains, technological for the development of dairy starter cultures and a number of functional dairy products, biochemical for evaluation of the nutritional and biological value of the products, and clinical trials. The implementation of the developed laboratory technologies on an industrial scale and the positive results obtained are of particular importance. All listed methods are modern, highly sensitive and distinguished by high reliability of the obtained results. Considering the complexity of the studied problem, the aim of the dissertation could hardly be fulfilled without their specific application.

4. Presentation of the obtained results.

The research was carried out at recognised scientific and educational organizations - Mongolian State University of Science and Technology, Moscow Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow State University of Applied Biotechnology, Konkuk University, South Korea, Japanese Research Laboratory, Agricultural University Plovdiv.

The submitted dissertation is presented in 288 standard pages. Its structure covers the following sections: Introduction, Analytical literature review, Aim and objectives, Organization, research objects and methods, Results and discussion, Conclusions and Contributions. The thesis contains 25 colour figures and 73 tables, and 257 references are cited. Most of them are from the past twenty years, with more than half of them published in the period 2010-2020. The candidate has presented a list of scientific publications based on the thesis, a list of patents, author's certificates and participation in research projects. In my opinion, the dissertation fully meets the requirements of the Law on the development of the academic staff of Republic of Bulgaria and the Regulations of the Agriculture University for its application with regards to this criterion.

5. Discussion of results and literature references

In the literature review, the candidate presents up-to-date information and analyzes the literature sources in several aspects: medico-biological basis for the development of functional food products, the current state of goat and sheep breeding in Mongolia, physico-chemical and microbiological characteristics of raw milk, scientific and technological aspects in the development of functional dairy products, prospects for the application of sheep and goat milk

for the production of dairy products on an industrial scale, features of traditional dairy products, microflora of probiotic products. The main characteristics of lactic acid bacteria - taxonomic, morphological and biochemical - have been examined.

Data on the proven positive effect on human health when taking probiotic food products are presented. The doctoral candidate demonstrates a wide knowledge on the literature materials concerning research on the chemical and microbiological status of raw sheep and goat milk, the molecular genetic methods for the identification of the isolated lactic acid bacteria and proving their probiotic characteristics.

My recommendation is that a summary of the analyzed literature is made at the end of the literature review, which will provide the basis and guidelines for the correct formulation of the aim and objectives of the dissertation.

Section "Results and Discussion" is the main part of the dissertation. It is well illustrated with tables and figures. The doctoral candidate has presented the performed experimental work with great precision and an excellent analytical approach. The research results are divided into five chapters, which logically follow the objectives set to achieve the aim of the dissertation work.

The first chapter "Overall resource of milk, determination of physico-chemical indicators and safety of milk from some farm animals characteristic of Mongolia" presents statistical data from 2020 on the number of goats and sheep bred in Mongolia and the volumes of processed sheep and goat milk. Amounts of processed milk are impressive - 259.6 million liters of goat and 165.6 million liters of sheep's milk, but it is reported that these volumes are significantly lower compared to cow's milk. Regarding the content of essential amino acids, no significant differences were recorded in the three studied milks, with values of 43.64%, 45.41%, and 44.49% for goat, sheep and cow's milk, respectively. Sheep's milk has been shown to contain the highest concentrations of calcium, sodium and magnesium. It is also high in vitamins B1 and B2. Milk safety was assessed by investigating the concentrations of heavy metals, pesticides and radionucleotides. The obtained results confirm the nutritional value and safety of goat and sheep milk as raw materials for the production of dairy products.

The results presented in the second chapter "Isolation and identification of microorganisms from national dairy products" are related to the isolation of lactic acid bacteria from 88 samples of traditional dairy products. More than 540 isolates were obtained and identified. Most of them were attributed to the species *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, and *Streptococcus thermophilus*. All strains were screened for probiotic characteristics and selected strains were deposited in the Mongolian National Collection of Microorganisms.

The third chapter "Selection of lactic acid bacteria strains and justification of methods for the development of bacterial starter cultures for obtaining functional dairy products", presents the research work continued with 14 strains belonging to the species *L. paracasei* subsp. *paracasei*, *L. paracasei* subsp. *tolerans*, *L. delbrueckii* subsp. *lactis*, *Lp. plantarum*, *L. paracasei*, *L. fermentum*, *Str. salivarius* subsp. *thermophilus*, *L. helveticus*, *L. delbrueckii* ssp. *bulgaricus*. After investigating pure cultures of lactic acid bacteria, the doctoral candidate

logically continued her work towards developing mixed starter cultures and proved that the combination of strains of *Lactobacillus paracasei subsp. paracasei* 06TSD19 and *L. paracasei subsp. tolerans* 06TSD39 yields a higher concentration of living cells and is characterized by a gentler product consistency and more pleasant taste and aroma. The technological studies showed that strain *L. fermentum* DTS/143 isolated from traditional products also showed good quality and was, therefore, selected for further research work with the aim of developing mixed starter cultures.

The fourth chapter "Development of technologies for the production of probiotic, synbiotic and protein products from goat, sheep and cow's milk" aims to provide scientifically proven data for the development of functional dairy products new to Mongolia, which could present a valuable tool for the prevention of a number of diseases. Results obtained in development of fermented dairy products from goat's milk are presented and the products are shown to contain high cell concentration and low acidity, which improves their sensory characteristics.

Based on these results, the main technological parameters for the production of fermented dairy products from goat's milk may be defined. The research results related to the development of synbiotic dairy products prove that the simultaneous introduction of fructooligosaccharides and inulin has a positive effect on the functional, structural-mechanical, sensory and microbiological indicators of the dairy products.

In the last chapter, the author presents the results of conducting *in vitro* and *in vivo* studies with the selected strains and the products obtained with them. When studying the anti-*Helicobacter* activity, the strains were cultivated for 48 hours in laboratory conditions together with strain *Helicobacter pylori* No. 130. The high concentration of L-lactic acid produced at the beginning of the cultivation significantly reduced the concentration of *Helicobacter pylori* cells at the beginning of the process. It was found that the growth inhibition of *Helicobacter pylori* when co-cultivated with *Lactobacillus paracasei subsp. paracasei* 06TSD19b is due not only to the action of organic acids, but also to other metabolites produced by the lactic acid bacteria. During *in vivo* studies with laboratory mice, they were first infected with *Helicobacter pylori*, which colonized the digestive tract, and then a culture of selected lactic acid bacteria was orally administered to the mice. The study shows that the cells of *Lactobacillus paracasei subsp. paracasei* 06TSD19b adhere to and colonize the epithelial tissue of the stomach, eliminating *Helicobacter pylori* cells from the body of mice.

By applying the double-blind testing method, clinical studies were conducted with a dairy product obtained by using strain *Lactobacillus paracasei subsp. paracasei* 06TSD19b. The target group consisted of 46 women aged 18 to 39 who took the product in certain doses for three weeks. The fecal analysis showed a higher concentration of L-lactic acid and a change in the composition and structure of the microbiome in the group that consumed the dairy product. In addition, RAPD analysis demonstrated the presence of live cells of the studied strain. This important result confirmed the ability of the strain to survive the passage through the entire digestive system and exhibit its probiotic properties.

In conclusion, my opinion is that the author is well familiar with the state of the art of the field studied and presented in the doctoral work. The research results are presented with competence and thoroughness, characteristic of a scientist who explored the dissertation topic

over a long period of time.

6. Dissertation Contributions

Based on the overall research results, the author has formulated six contributions - five of a scientific nature and one with applied nature.

In my opinion, the following scientific contributions are of highest significance:

- By using molecular method, strains *Lactiplantibacillus plantarum* and *L. paracasei* spp were identified, and their probiotic characteristics were further demonstrated.
- It has been proven that strain *L. paracasei* spp. *paracasei* 06TSD19b isolated from traditional dairy products has pronounced anti-helicobacter and probiotic characteristics.
- The anti-helicobacter and prophylactic effectiveness of the obtained functional dairy products has been demonstrated in clinical studies

Applied contributions include creating a culture collection of isolated lactic acid bacteria from traditional dairy products and developing starter cultures with some of the strains.

7. Critical notes and questions

- In 2020, a new taxonomy of lactic acid bacteria species was published, with changes in the names of a number of species. In the dissertation work, the species of lactic acid bacteria are presented with the outdated names, which is probably due to the use of the names in previous publications of the doctoral candidate.
- In my opinion, an analytical summary of the main findings from the used literature should be made at the end of the literature review. This would enable the candidate to make a correct assessment of the current state of the studied problem and to define the aim and objectives of the dissertation work, which would build on the current state of the art of the research field.
- Have the yeasts isolated from traditional dairy products been identified? (p. 238)
- Are there any differences between strains *Lactobacillus paracasei* subsp. *paracasei* 06TSD19b and *Lactobacillus paracasei* subsp. *paracasei* 06TSD19b?

Published articles and citations

The doctoral candidate presents 36 scientific publications on the dissertation work, 10 of which are in refereed scientific journals. The candidate has participated in 8 research projects with national and international funding. She has written three monographs and co-authored seven patents (authorship certificates are presented). Until now, 107 citations of the author's publications have been registered in the Scopus platform. According to this indicator, the doctoral candidate fulfills the requirements of the Law on the development of the academic staff of Republic of Bulgaria and the Regulations of the Agriculture University for its application.

The dissertation abstract reflects objectively the structure and content of the dissertation

work, with clearly presented main ideas, results and conclusions of the experiments

CONCLUSION

Based on the presented materials with different research methods learned and applied by the doctoral candidate, correctly performed experiments, summaries and conclusions made, publications and overall expertise built by the candidate, my opinion is that the dissertation meets the requirements of the Law on the development of the academic staff of Republic of Bulgaria and the Regulations of the Agriculture University for its application, which provides the grounds for my POSITIVE evaluation.

Therefore, I would like to propose to the honorable Scientific Jury to vote positively and to award Assoc. Prof. Dr. Tsend-Ayush Chuluunbat the scientific degree "Doctor of Sciences" in scientific major "Technology of milk and dairy products".

Date: 15.09.2022 r.
Plovdiv, Bulgaria

REVIEWER:


Prof. Dr. Angel Angelov