



**THE POTENCIAL TOLERANCE OF PROBIOTIC DADIAH TO ZINC  
“NOVEL OF OBESITY THERAPY”**

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**Abstract**

*Dadiah is included in functional food because it contains lactic acid bacteria (LAB) which have the potential to be probiotics. The combination of foods containing bacteria with other compounds must be treated so that they do not inhibit or kill lactic acid bacteria. Zinc as one of the micronutrients that is important for the body and is also an antimicrobial but has benefits for obese people. Giving LAB with zinc should provide support to each other without reducing the impact of any of the materials used. This study aims to determine the tolerance of probiotic potential lactic acid bacteria to zinc so that zinc resistant LAB isolate is obtained to be applied to obese patients. To determine the correlation and relationship of zinc and LAB, observations were made on the microscopic and macroscopic characteristics of LAB from Bukittinggi drugs such as fermentation type, catalase test, gram staining and zinc resistance using the welling method and total colony. The results obtained in the study obtained 5 isolates from Bukittinggi curd, all isolates including homofermentative fermentation types, negative catalase tests and including Gram Positive results from gram staining. LAB resistance to zinc using the well method did not obtain clearzone for all isolates and LAB resistance to zinc obtained viability below 20% for all isolates. In the research that has been done, it was found that LAB isolated from Bukittinggi drug has characteristics that have potential as probiotics and are also resistant to zinc so that the combination of LAB and zinc for obese patients can be carried out to the next stage.*

**Keywords:** *Dadiah, Lactic acid bacteria, Probiotic, Zinc*

**INTRODUCTION**

Food and Agriculture Organization (FAO) and the World Health Organization define probiotics as microorganisms that exist in the host's body in sufficient numbers to benefit those who ingest them. By functioning anaerobically, probiotics can generate lactic acid bacteria, which lowers the pH of the digestive tract and prevents the growth of harmful bacteria. Curd isolation is one potential source of probiotics. Dadiah is a common dish from the West Sumatra region. It is made by fermenting buffalo milk naturally using microorganisms that produce the lactic acid that is naturally present in buffalo milk in bamboo tubes (Purwati *et al.*, 2016). Fresh buffalo milk is poured into bamboo sticks, which are then wrapped with banana leaves and left to ferment

for two days at room temperature, according to Surono (2000). Dadiah is a source of lactic acid bacteria growth since it has a complete nutritional makeup. According to Purwati *et al.* (2016), lactic acid bacteria (LAB) are a kind of bacteria that can convert lactose, a type of carbohydrate, into lactic acid. Because it has a whole range of nutrients and is good for health, curd can be utilized as a source of probiotics.

The genus *Lactobacillus* belongs to lactic acid bacteria, which is a group of bacteria that can ferment carbohydrates into lactic acid and benefit its host (Cynthia *et al.*, 2015). The development of LAB identification continues to grow; some research results found LAB from dadiah such as *Lactobacillus plantarum* (Harun *et al.*, 2020; Roza *et al.*, 2022; Abdullah *et al.*, 2021), *Lactobacillus fermentum* (Amelia *et al.*, 2020), *Pediococcus acidilactici* (Dewi *et al.*, 2023), *Lactococcus lactis* (Sukma *et al.*, 2019).

Zinc, one of the most important and abundant elements in the human body, is closely related to human health (Gammoh & Rink, 2017). Zinc is an indispensable micronutrient for various functions in the organism: zinc is required for cell proliferation and differentiation, DNA synthesis, and RNA transcription, as well as maintaining homeostasis (Lothar, 2000; Michalczyk & CymLABuk-Poska, 2020); zinc is required for stabilization of protein structures and activation of many enzymes as zinc can act as a structural ion and cofactor for more than 300 enzymes such as alcohol dehydrogenase, copper-zinc superoxide dismutase and RNA polymerase (Beyersmann, 2002; Chasapis *et al.*, 2012); zinc also plays a role in oxidative stress, cellular aging and apoptosis as well as immune response as zinc has been shown to have antioxidant and anti-inflammatory activities (Michalczyk & CymLABuk-Poska, 2020; Stefanidou *et al.*, 2005). Zinc deficiency can lead to the development and progression of various chronic and inflammatory diseases (including cancerogenesis, neurodegeneration, atherosclerosis, cardiovascular diseases, and immunological diseases) as well as some infectious diseases such as measles, malaria, and pneumonia (Gammoh & Rink, 2017; March 2013).

According to research, enough zinc supplementation from other sources is also required, especially for pregnant women and young children, in addition to naturally occurring foods rich in zinc. However, due to their high cost and the fact that the body cannot completely absorb inorganic zinc, medications containing organic zinc have not been utilized frequently. Yeast is one example of a microorganism that has been employed to transport trace elements and has positive benefits (Guo *et al.*, 2020). Some lactic acid bacteria, including *L. plantarum* and *L. acidophilus*, have been shown to be able to internalize ferrous iron and oxidize it into ferrous form, according to Kot, Furmanov, and Bezkorovainy (2010). Additionally, it has been noted that *L. plantarum* and *L. brevis* have a high affinity for copper and zinc binding. Metal ions can either be transported and stored inside cells or bound to their cell surface (Mrvcic *et al.* 2012). It has an impact on the physiological and biological functions of lactic acid bacteria. The development and production of acid by lactic acid bacteria have been demonstrated to benefit from the addition of copper (Mrvcic, 'Soli'c, and Butorac, 2016). Considering that zinc and lactic acid bacteria are crucial for regulating a range of bodily physiological processes. Additionally, the addition of zinc-enriched probiotics to the body has the potential to alter both the lactic

acid bacteria's probiotic activity as well as the body's requirement for zinc, so that isolates and types of LAB that have resistance to zinc are obtained. This study aims to determine the resistance of LAB to zinc so as to get LAB that supports its host when combined with zinc for obese patients.

## **MATERIAL AND METHOD**

Dadiah samples were taken from farm in Bukittinggi City which were then taken using a cool box and stored at the Biomedical Laboratory, Baiturrahmah University for identification analyze.

### **Research design and analysis**

This research used a descriptive experimental design with duplicate work.

### **Identification and Isolation of Lactic Acid Bacteria**

Dadiah, weighing approximately 1 gram, was after placed into a test tube containing 9 ml of de Mann Ragosa Sharpe (MRS) Broth (MERCK , Germany) solution. The contents were then subjected to vortexing until achieving a state of homogeneity. The outcome is referred to as a 10-1 dilution, which is, after that, subjected to a 24 h incubation period in a 37°C incubator (Mettler DI 55). The diluted sample, consisting of 100 µl, was transferred into an Eppendorf tube containing 900 µl of Demann Rogosa Sharpe (MRS) Broth MERCK solution. Subsequently, a serial dilution was performed, resulting in a dilution factor of 10-8. A volume of 100 µL was extracted from a dilution of 10-8 and afterward inoculated into a petri dish with MRS Agar media (MERCK) using the spread method. The surface of the agar was then leveled using a hockey stick. The inoculum was placed in an anaerobic jar and, after that, subjected to incubation in a controlled environment for 48 hours at a temperature of 37°C. Following a 48 h incubation period, the solitary colony that exhibited the typical characteristics of lactic acid bacteria, specifically a smooth, round, yellowish-white appearance, was transferred onto MRS Agar media (MERCK, Germany) for the purpose of purifying the colony. This was achieved using the streak method, employing an aseptic needle, and subsequently incubating the culture for 24 h at a temperature of 37°C (Purwati *et al.*, 2015).

### **Biochemical Properties Test Catalase Test**

The catalase test was conducted using isolates of lactic acid bacteria obtained by aseptic techniques with the use of an inoculation needle. The specimen is gently scraped onto the surface of the glass slide. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) droplets were dispensed using a 50-liter pipette. The examination of bacterial reviews involves the monitoring of gas formation (Public Health Englad, 2014).

### **Type Fermentation Test**

The lactic acid bacteria obtained were subjected to fermentative-type testing. This was done by inoculating the lactic acid bacteria isolate into 5 ml of MRS Broth (MERCK, Germany), followed by the placement of a Durham tube in an inverted position. The sample should be subjected to incubation for 24 h at a temperature of 37°C. The act of visually examining the Durham tube and noting the presence or absence of air bubbles is a means of making observations (Suryani *et al.*, 2010).

### Gram-staining

Use method Indo Reagen, culture LAB was obtained and transferred onto a Petri dish using a sterile needle. The culture was then transferred onto a glass slide. Violet crystals were applied to the culture and left for 1 min. The slide was subsequently rinsed with distilled water and dried. Drops of iodine were added to the slide and left for 1 min, followed by another rinse with distilled water and drying. The slide was then immersed in ethanol for approximately 20 min. Finally, a single drop of safranin was added to the slide and left for 30 s. The slide was rinsed and dried, and the bacterial morphology was observed using a microscope.

### Zinc Resistance

LAB resistance test against zinc using agar well diffusion method. MRS Agar media, as much as 20 ml was added with 0.2% rejuvenated lactic acid bacteria, allowed to harden in a petri dish. Then holes were made in the MHA media with a diameter of  $\pm 6.5$  mm using a cork barrier. Next, 50  $\mu$ l of zinc that has been dissolved with distilled water is injected. After that, it was incubated at 37°C. Observations were made of the clear zone in the shape of a circle after 48 h using a push-pull term (Yang and Ranalla, 2012).

### Enrichment Lactic Acid Bacteria with Zinc

Culture LAB was grown in MRS medium for 18 h at 37 °C and then centrifuged at 4 °C (2000 $\times$ g, 10 min). The collected strains were placed in an aqueous solution of zinc sulfate with a concentration of 400 mg/L, and cultured with shaking at 37 °C for 1 h, followed by centrifugation-washing-centrifugation to obtain zinc-enriched LAB culture and without zinc enrichment was used as the control strain (Yue meng *et al.*, 2022).

## RESULT AND DISCUSSION

### Total Lactic Acid Bacteria

The lactic acid bacteria colony was counted using a Quebec colony counter and obtained a total of  $46 \times 10^9$  CFU/gr LAB colonies. The results obtained were in accordance with FAO/WHO (2001), the total number of LAB colonies in curd as a LAB probiotic food produced was  $10^6 - 10^8$  CFU/g. The results of macroscopic observations from single colony LAB on dadiah can be seen in Table 1.

**Table 1. Macroscopic Identification Single Colony**

Sample	Colour	Shape	Surface
DB1	White-yellow	round	Smooth, convex
DB2	White-yellow	round	Smooth, convex
DB3	White-yellow	round	Smooth, convex
DB4	White-yellow	round	Smooth, convex
DB5	White-yellow	round	Smooth, convex

Macroscopic observation of LAB found creamy white colonies, round in shape with smooth edges and a convex surface on MERCK MRS Agar media, which are the characteristics of colony shapes resulting from the identification of lactic acid bacteria (LAB) isolated from samples DB1, DB2, DB3, DB4, and DB5. In accordance with research conducted by Purwati *et al.* (2005), lactic acid bacteria colonies grown on MRS Agar media were creamy white.

**Biochemical Test**

**Table 2. Result Type Fermentation And Catalase Test**

Isolate	Type Fermentative	Catalase Test
DB1	Homofermentative	Negative
DB2	Homofermentative	Negative
DB3	Homofermentative	Negative
DB4	Homofermentative	Negative
DB5	Homofermentative	Negative

From Table 2, all isolates from this study obtained negative results (-); observations were made by looking at the presence or absence of gas bubbles in the isolates that had been dripped with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Catalase is said to be positive if gas bubbles are found in the isolate that has been dripped with hydrogen peroxide. The general LAB criteria that have been carried out are that bacteria must react negatively to catalase. This catalase test is carried out to determine whether or not there is activity of the catalase enzyme in bacteria, where the catalase enzyme will play a role in breaking down hydrogen peroxide into water (H<sub>2</sub>O) and oxide (O<sub>2</sub>). In accordance with research by Pratama *et al* (2021), lactic acid bacteria, which have the potential to act as probiotics, do not produce the enzyme catalase.

Fermentation Test is one of the biochemical properties tests. The purpose of this test is to classify LAB into the homofermentative group or heterofermentative group. In Table 2, it can be seen that the five isolates used in this study were of the homofermentative fermentation type. When observed using a Durham tube, no bubbles were found. The group of lactic acid bacteria is generally of the homofermentative type and produces the main product during the fermentation process, lactic acid. Suryani, Santoso, and Jufrie (2010) stated that there are two types of lactic acid bacterial fermentation, namely homofermentative and heterofermentative. Homofermentative lactic acid bacteria only produce lactic acid as the main product of their fermentation. At the same time, heterofermentative lactic acid bacteria, besides lactic acid, also produce ethanol, other acids such as acetic acid and CO<sub>2</sub> gas, so if the lactic acid bacteria tested produce gas which is stored in a Durham tube, the bacteria The lactic acid is declared heterofermentative. In contrast, isolates that do not produce or produce gas are called homofermentative.

**Gram-staining**

The research obtained results for gram-positive bacteria (purple) with rod shapes (bacilli). The Gram staining results of LAB from the five samples were purple, so LAB was categorized as Gram-positive bacteria with rod morphology (bacilli). In accordance with the opinion of Salminen, Wright, and Ouwehand (2004), which states that gram-positive, rod-shaped, or round, facultatively anaerobic bacteria do not form spores and produce lactic acid bacteria as the main product of carbohydrate fermentation (glucose, fructose, and sucrose). Characteristics of LAB research conducted by Aritonang *et al.* (2022) also obtained lactic acid bacteria from Gram- positive and rod-shaped Lintau dadiah.

**Zinc Resistance**

**Table 3. Viability and Zinc Resistance Lactic Acid Bacteria**

ISOLATE	Viability (%)	Clear Zone	
		Zinc Tablet	Zinc capsule

DB1	90,28	clear	clear
DB2	84,24	clear	clear
DB3	87,43	clear	clear
DB4	88,35	clear	clear
DB5	85,53	clear	clear

In Table 3, it can be seen that in the LAB resistance test to zinc, no clear zones were found, meaning that zinc did not inhibit the growth of LAB on MRS agar. Likewise, the viability obtained by all isolates had a viability of >80%, meaning that the higher the viability obtained, the better the resistance of a microorganism. Similar findings were also obtained by Sun and Shao (2007) in research on the adsorption of lead ions by lactic acid bacteria. Additionally, oral supplementation with Zn-enriched *L. plantarum* CCFM242 has been reported to reveal higher serum zinc levels and exhibit stronger modulatory effects on antioxidant and immune defense systems in the intestine of normal mice compared with ZnSO<sub>4</sub> or *L. plantarum* CCFM242 treatment only (Zhai *et al.*, 2019). Our results show that *L. plantarum* DNZ-4 can interact with zinc ions in aqueous solution and enrich zinc ions through its physiological function, indicating that *L. plantarum* DNZ-4 can be used as an efficient carrier for zinc delivery. In addition, the TetR protein in LAB is a widespread family of one-component systems (OCS) that regulates many aspects of bacterial physiology and interacts with a variety of ligands. A small number of TetR family regulators have been characterized for LAB. Resistance to zinc is largely dependent on the CzcD transporter, whose expression is under the control of SczA and whose activity is dependent on Zn<sup>2+</sup>. Despite extensive research on other TetR regulators, metal-binding TetR regulators are rare in bacteria and await further characterization (Bao *et al.*, 2010).

LAB developed mechanisms to tolerate zinc by preventing its toxicity. Some lactobacilli and bifidobacteria have been reported to be resistant to various metal ions at different concentration levels; some of them show the capacity to tolerate up to 100-500 extracellular zinc (Yusof *et al.*, 2020; Leonardy *et al.*, 2013). To combat zinc toxicity, LAB can induce the expression of a zinc efflux system most likely encoded by the *czcD* or *cadA* genes (Papadimitriou *et al.*, 2016; Huynh *et al.*, 2022). In addition, they limit the amount of zinc available through surface biosorption and the formation of extracellular ZnO nanoparticles (Yusof *et al.*, 2020; Mrvacic *et al.*, 2012). The tolerance of *Fructilactobacillus sanfranciscensis* DG1 to zinc may be due to one of these mechanisms or even a combination of both. When fermented in mMRS broth, *Fructilactobacillus sanfranciscensis* DG1 contained approximately 0.032 fg Zn/cell (~300,000 zinc atoms per cell), which is very close to the experimentally determined “minimum zinc content” in prokaryotes (105 zinc atoms per *Escherichia coli* cell). According to existing evidence, minimum zinc levels are likely determined by the number of zinc-binding sites present on proteins important for optimal cell function (Wang *et al.*, 2018). According to our data, *Fructilactobacillus sanfranciscensis* DG1 appears to have a moderate and homeostatically LABanced zinc requirement despite a high extracellular zinc load. Lactic acid bacteria (LAB), as important food-grade bacteria with probiotic advantages, have been studied extensively. Only a few studies on Zn-enriched LAB have been conducted, but it was found that bacterial growth and the probiotic effects of *L.*

*plantarum* can be enhanced by zinc in the intestine. Moreover, each genus and strain of LAB has different resistance depending on the source of the isolate, so before preclinical and clinical testing it is necessary to conduct in vitro analysis of LAB resistance to zinc.

## CONCLUSION

Dadiah Bukittinggi possesses a total LAB count of  $46 \times 10^9$  CFU/gr. Based on the overall count of LAB colonies, a subset of five individual colonies was chosen for subsequent analysis. Subsequent investigation revealed that all five isolates exhibited characteristics consistent with the homofermentative fermentation type, as proven by negative findings in the catalase test. The resistance of the five isolates to zinc was seen using the pure plate welling method; no clear zones were found on MRS Agar, and the viability value obtained for LAB enrichment with zinc was in the range of 84.42-90.28%. From this study, it was found that LAB isolate from Bukittinggi drug has the ability to withstand zinc so that it can be combined for obese patients for the next study.

## REFERENCE

- Abdullah D, Poddar S, Rai RP, Purwati E, Dewi NP, Pratama YE. (2021). Molecular Identification of Lactic Acid Bacteria an Approach to Sustainable Food Security. *Journal of Public Health Research*, 10 (2suppl).
- Amelia,R, Philip,K, Pratama, Y.E, Purwati, E. 2020. Characterization and probiotic potential of lactic acid bacteria isolated from *dadiah* sampled in West Sumatra. *Food Science and Technology*, 41, 746-752.
- Aritonang SN, Roza E, Yetmaneli, Sandra A, Rizqan. (2022). Characterization of Lactic Acid Bacteria from Buffalo Dairy Product (Dadiah) as Potential Probiotics. *Biodiversitas*, 23, 4418-4423.
- Bao, B., Prasad, A. S., Beck, F. W., Fitzgerald, J. T., Snell, D., Bao, G. W., Singh, T., & Cardozo, L. J. (2010). Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. *The American Journal of Clinical Nutrition*, 91 (6), 1634-1641.
- Dewi, N.P, Poddar,S, Rai, R.P, Purwati, E. Abdullah, D, Pratama Y.E. (2023). The Potential Lactic Acid Bacteria from *Dadiah* Sianok Bukittinggi City, West Sumatera as Probiotic. *Research Journal of Pharmacy and Technology*, 16 (2), 627-634.
- FAO/WHO. (2006). Probiotics in Food: Health and Nutritional Properties and Guidelines for evaluation. *FAO Food and Nutrition*, 85.
- FAO/WHO. (2001). *Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food*. London.
- Gammoh, N. Z., & Rink, L. (2017). Zinc in infection and inflammation. *Nutrients*, 9 (6), 624.
- Guo, H., Guo, S., & Liu, H. (2020). Antioxidant activity and inhibition of ultraviolet radiation- induced skin damage of Selenium-rich peptide fraction from selenium-rich yeast protein hydrolysate. *Bioorganic Chemistry*, 105, 104431.



- Huynh, U.; Qiao, M.; King, J.; Trinh, B.; Valdez, J.; Haq, M.; Zastrow, M.L. (2022). Differential Effects of Transition Metals on Growth and Metal Uptake for Two Distinct *Lactobacillus* Species. *Microbiol. Spectr.* 10, e0100621.
- Kang, S., Li, R., Jin, H., You, H. J., & Ji, G. E. (2020). Effects of selenium- and zinc-enriched *Lactobacillus plantarum* sezi on antioxidant capacities and gut microbiome in an icr mouse model. *Antioxidants*, 9 (10), 1-14.
- Kot, E., Furmanov, S., & Bezkorovainy, A. (2010). Accumulation of iron in lactic acid bacteria and bifidobacteria. *Journal of Food Science*, 60 (3), 547-550.
- Leonardi, A.; Zanoni, S.; De Lucia, M.; Amaretti, A.; Raimondi, S.; Rossi, M. (2013). Zinc Uptake by Lactic Acid Bacteria. *ISRN Biotechnol*, 312917.
- Lothar, R. (2000). Zinc and the immune system. *Proceedings of the Nutrition Society*, 59 (4), 541–552.
- Maret, W. (2013). Zinc and human disease. *Metal ions in life sciences*, 13, 389-414.
- Meng, Y., Liang, Z., Yi, M., Tan, Y., Li, Z., Du, P., & Liu, L. (2022). Enrichment of zinc in *Lactobacillus plantarum* DNZ-4: Impact on its characteristics, metabolites and antioxidant activity. *LWT*, 153, 112462.
- Michalczyk, K., & CymLABuk-Poska, A. (2020). The role of zinc and copper in gynecological malignancies. *Nutrients*, 12 (12), 3732.
- Mrvčić, J., Stanzer, D., Šolić, E., & Stehlik-Tomas, V. (2012). Interaction of lactic acid bacteria with metal ions: Opportunities for improving food safety and quality. *World Journal of Microbiology and Biotechnology*, 28 (9), 2771-2782.
- Mrvčić, J.; Stanzer, D.; Šolić, E.; Stehlik-Tomas, V. (2012). Interaction of lactic acid bacteria with metal ions: Opportunities for improving food safety and quality. *World J. Microbiol. Biotechnol.* 28, 2771-2782.
- Papadimitriou, K.; Alegría, Á.; Bron, P.A.; de Angelis, M.; Gobbetti, M.; Kleerebezem, M.; Lemos, J.A.; Linares, D.M.; Ross, P.; Stanton, C. (2016). Stress Physiology of Lactic Acid Bacteria. *Microbiol. Mol. Biol. Rev.* 80, 837-890.
- Pratama Y. E., Jamsari, Yuherman, Melia S., Purwati E. (2021). Molecular identification of lactic acid bacteria from “budu” of West Sumatera, Indonesia, as a potential probiotic. *AAFL Bioflux*, 14 (5), 2837-2844.
- Public Health England. (2014). Identification of *Staphylococcus* Species, *Micrococcus* Species and *Rothia* Species. UK Standards for Microbiology Investigations, UK.
- Purwati, E., S. Syukur dan Hidayat, Z. (2005). *Lactobacillus* sp. Isolasi dari *Biovicophitomega* sebagai Probiotik. Jakarta: Lembaga Ilmu Pengetahuan Indonesia.
- Roza E, Aritonang SN, Yellita Y, Susanty H, Rizqan, Pratama YE. (2021). Potential of dadiah kapau from Agam District, Indonesia as a source of probiotics for health. *Biodiversitas*, 23, 564-571.
- Salminen, S., & Von Wright, A. (Eds.). (2004). *Lactic Acid Bacteria: Microbiological and Functional Aspects* (Vol. 139). CRC Press.



- Sukma, A., T.T.T Nguyen., N. Fitria, I. Mimura., R. Kaneko., K. Arakawa., dan H. Morita. 2017. Microbiota Community Structure in Traditional Fermented Milk *Dadiah* in Indonesia: Insight from High-throughput 16S rRNA Gene Sequencing. *Dairy Microbiology*, 7: 1-3.
- Sun, F., & Shao, Z. (2007). Biosorption and bioaccumulation of lead by *Penicillium* sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean. *Extremophiles*, 11 (6), 853-858.
- Surono, I. S. 2004. *Probiotik Susu Fermentasi dan Kesehatan*. Jakarta: Yayasan Pengusaha Makanan dan Minuman Seluruh Indonesia (YAPMMI).
- Suryani Y, Astuti, Oktavia B, Umniyati S. (2010). Isolasi Dan Karakterisasi Bakteri Asam Laktat Dari Limbah Kotoran Ayam Sebagai Agensi Probiotik Dan Enzim Kolesterol. *Prosiding Seminar Nasional Biologi*, 138-147.
- Wang, Y, Weisenhorn, E.; MacDiarmid, C.W.; Andreini, C.; Bucci, M.; Taggart, J.; Banci, L.; Russell, J.; Coon, J.J.; Eide, D.J. (2018). The cellular economy of the *Saccharomyces cerevisiae* zinc proteome. *Metallomics*, 10, 1755-1776.
- Yang, Ziheng, Bruce Rannala. (2012). Molecular Phylogenetics: Principles and Practice. *Nature Reviews Genetics*, 13, 303-314.
- Yusof, H.M.; Mohamad, R.; Zaidan, U.H.; Rahman, N.A. (2020). Sustainable microbial cell nanofactory for zinc oxide nanoparticles production by zinc-tolerant probiotic *Lactobacillus plantarum* strain TA4. *Microb. Cell Factories*, 19, 10.
- Zhai, Q., Zhang, Q., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2019). The synergistic effect of *Lactobacillus plantarum* CCFM242 and zinc on ulcerative colitis through modulating intestinal homeostasis. *Food & Function*, 10 (9), 6147-6156.