TẠP CHÍ KHOA HỌC & CÔNG NGHỆ ĐẠI HỌC DUY TÂN
DTU Journal of Science and Technology 5(60) (2023) 114-122

TRƯỜNG ĐẠI HỌC DUY TÂN
DUYTAN UNIVERSITY

Review: Isolation and identification of Bifidobacteria from commercial yoghurt and its applications in Vietnamese ginseng biotransformation

Tổng quan về phương pháp phân lập và định danh Bifidobacteria từ sữa chua thương mại và ứng dụng trong chuyển hóa sinh học sâm Ngọc Linh

Nguyen Thuy Cat Dung[a], Trinh Minh Anh[a], Le Quang Phong[a*]
Nguyễn Thuỳ Cát Dung[a], Trịnh Minh Anh[a], Lê Quang Phong[a*]

[a]*International Univerity, Vietnam National University of Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam*
[a]*Trường Đại học Quốc tế, Đại học Quốc gia Hồ Chí Minh, TP. Hồ Chí Minh, Việt Nam*

(Ngày nhận bài: 29/8/2023, ngày phản biện xong: 06/10/2023, ngày chấp nhận đăng: 15/10/2023)

Abstract

Panax vietnamensis, also known as Ngoc Linh ginseng, contains a high ginsenoside content making it a widely consumed traditional herbal. The medicinal value of Ngoc Linh ginseng can be enhanced significantly by converting ginsenosides to rare ginsenosides by biotransformation using Bifidobacteria. The general procedure of fermenting ginseng by bacterial strains involves four main steps. This review aims to revise the methods of isolation and identification of Bifidobacteria from commercial yoghurt and its use in ginseng fermentation. Isolation methods for *Bifidobacterium* spp. include various selective media which is followed by the strain identification using Polymerase Chain Reaction and sequencing or carbohydrate fermentation patterns. The ginseng fermentation is assessed with Thin Layer Chromatography and High-Performance Liquid Chromatography. The identification confirms the successful isolation of *Bifidobacterium* spp. from fermented dairy products and the results of biotransformation rate assessment of this probiotic demonstrate new opportunities for enhanced ginsenoside conversion method.

Keywords: Vietnamese ginseng, biotransformation, Bifidobacteria.

Tóm tắt

Panax vietnamensis, còn được gọi là sâm Ngọc Linh, là một loại thảo dược truyền thống được tiêu thụ rộng rãi nhờ có hàm lượng ginsenoside cao. Giá trị dược liệu của sâm Ngọc Linh có thể được tăng cường đáng kể bằng cách sử dụng vi khuẩn Bifidobacteria để lên men nhân sâm nhằm chuyển đổi hoá sinh học ginsenosides. Quy trình tổng quát của quá trình lên men nhân sâm bằng các chủng vi khuẩn bao gồm bốn bước chính. Trong bài viết này, các phương pháp phân lập và xác định Bifidobacteria từ sữa chua thương mại cũng như việc sử dụng nó trong quá trình lên men sẽ được tổng hợp lại. Bước đầu tiên trong quy trình là phân lập *Bifidobacterium spp.* bao gồm sử dụng các môi trường chọn lọc khác nhau. Bước tiếp theo là định danh chủng vi khuẩn bằng cách sử dụng phản ứng chuỗi polymerase (PCR) và giải trình tự hoặc bằng cách xác định kiểu lên men carbohydrate. Quá trình lên men nhân sâm được đánh giá bằng sắc ký bản mỏng và sắc ký lỏng hiệu năng cao. Kết quả định danh chủng vi khuẩn cho thấy sự phân lập thành công *Bifidobacterium spp.* từ các sản phẩm sữa lên men và kết quả đánh giá tốc độ chuyển hóa sinh học của chế phẩm sinh học này cho thấy những cơ hội mới của phương pháp chuyển hóa ginsengnoside.

Từ khóa: Sâm Ngọc Linh; chuyển hoá sinh học; Bifidobacteria.

[*]*Corresponding Author:* Le Quang Phong
Email: lqphong@hcmiu.edu.vn

1. Introduction

Panax ginseng, a traditional herbal medicine, has been amassed global popularity as a treatment and prevention for various diseases owing to its prominent effects towards body inflammation reaction [1]. In Vietnam, this species is named *Panax vietnamenesis* which was found in 1973 in Ngoc Linh mountainous area, located between the provinces of Kon Tum and Quang Nam. In comparison to the well-known Korean ginseng, Ngoc Linh ginseng has been reported to contain a higher saponin contents in which the ginsenosides are the major bioactive constituents [2]. Vietnamese ginseng not only contains a high yield of majonosides but also has an array of different ginsenosides such as Rb1, Rd, Re and a unique vina-ginsenoside R1 and R2 [3]. Some of the structures of major ginsenosides and their metabolism pathway in human body are demonstrated in **Figure 1**. The deglycosylation of ginsenosides has been performed and their pharmaceutical effects are proved to be higher than the original majonosides. These beneficial enhancements can be explained by the deglycosylated ginsenosides' compact structure, higher bioavailability, superior ability to permeate through cell membrane [4]. Among different available approaches for the purpose of preparing a deglycosylated ginsenoside, fermentation method, also known as biotransformation using microbial cells, is more favorable thanks to its significant efficiency. Additionally, fermentation method offers high specificity and selectivity at a marginal cost with few negative impacts on the product's quality and environment compared to other methods [5].

There are various types of probiotics used in food industry to perform the deglycosylation of ginseng extract to enhance its biological activity. One of the highly suitable probiotic microorganisms are Bifidobacteria due to their vast health benefits, especially high glycosidase activity [6]. *Bifidobacterium* is a genus of gram-positive bacteria. They are nonmotile and typically branched who live in gastrointestinal tracts, vagina, and mouth of mammals [7]. The bacterial strains are also commonly utilized in the food industry to produce fermented dairy products such as yoghurt, thus, they are abundant and can be conveniently isolated for the purpose of the ginseng biotransformation. In this review, the *Bifidobacterium spp.* isolated from commercial yoghurt product and its applications in ginseng fermentation will be focused.

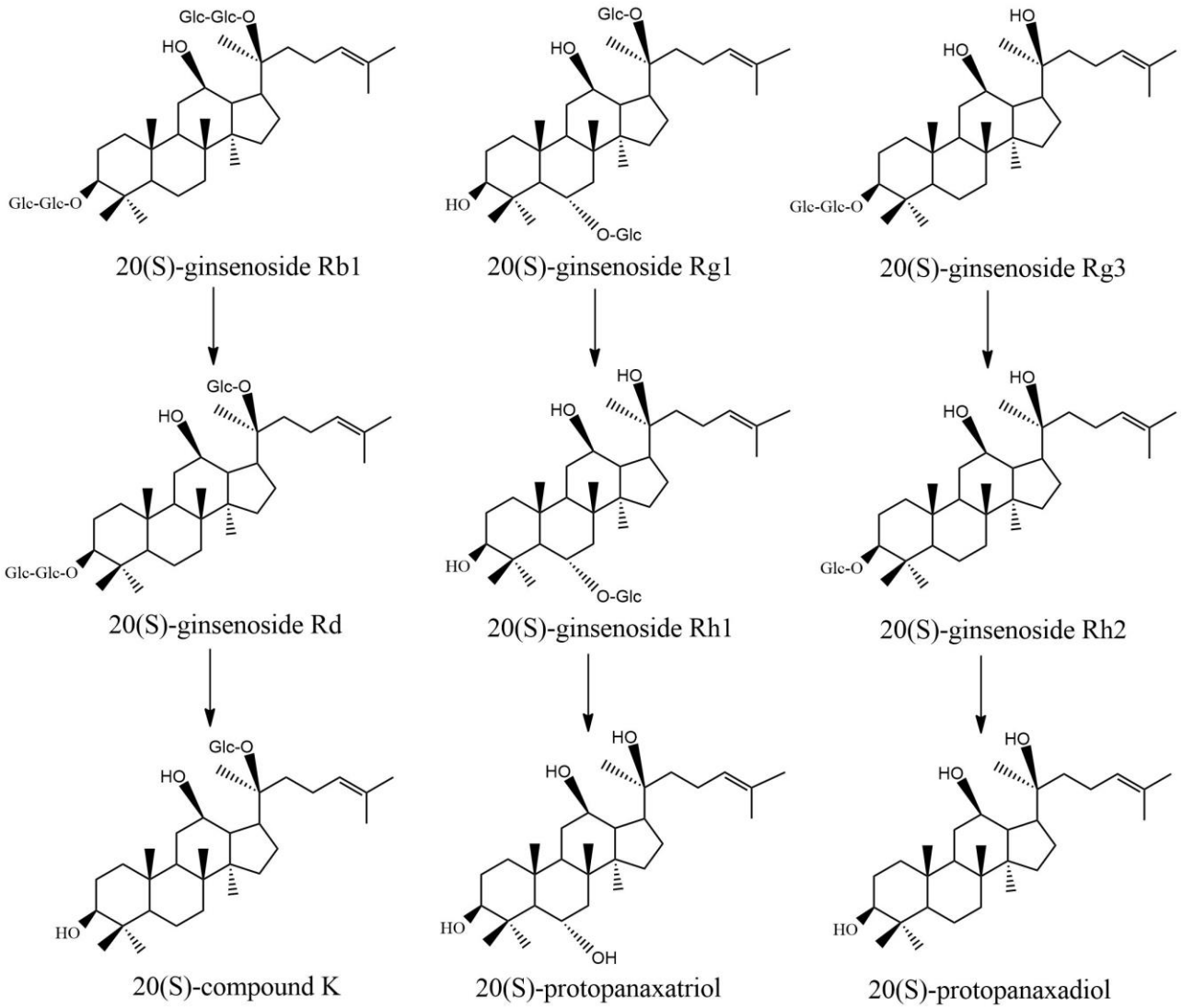

Figure 1. Major ginsenosides and the metabolism pathways of these substances in humane body [8].

The standardized ginseng fermentation process using *Bifidobacterium* strains consists of four main steps including the isolation of *Bifidobacterium* strains from fermented yoghurt, identification of the strains, biotransformation of ginsenosides, and assessment of biotransformation rate. This review aims to summarize different approaches for each step of the process as demonstrated in **Figure 2**. In the strain isolation stage, two selective media are commonly used involving De Man, Rogosa and Sharpe (MRS) agar fortified with L-cysteine and galactose agar fortified with lithium chloride. Then, the isolated strains can be identified using Polymerase Chain Reaction (PCR) and sequencing method or using their carbohydrate fermentation pattern analysis. Following the identification, the isolated strains are cultured for mass multiplication and used in the ginseng fermentation. In order to evaluate the in vitro conversion from majonosides to novel ginsenosides, Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) are utilized to provide assessment data.

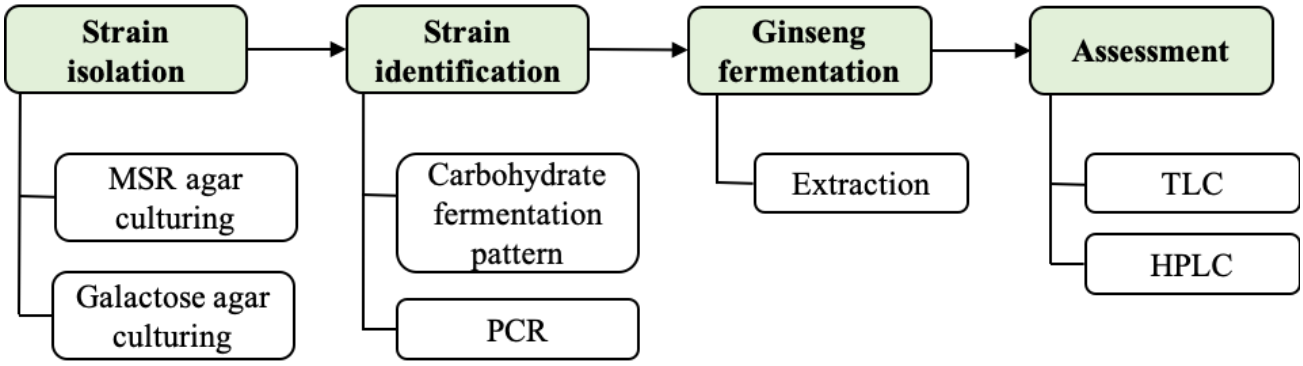

Figure 2. Schematic diagram for overview of ginseng fermentation process.

2. Isolation of *Bifidobacterium* strains from fermented yoghurt

Yoghurt is a common type of bacterial fermented dairy whose production relies on the starter culture of multiple bacteria strains, such as *Lactobacillus bulgaricus, Streptococcus thermophilus, Bifidobacterium lactis, Lactobacillus acidophilus* [9]. *Bifidobacterium* spp. existing in the human intestinal microbiome can be categorized into two groups. One group includes *B. breve, B. infantis,* and *B. bifidum* which can be found in neonates' intestine. The remaining group in the aldult's intestine contains *B. adolescentis* and *B. longum* [10]. Hence, it is crucial to effectively isolate *Bifidobacterium* strains from the yoghurt culture mixture to ensure the consistency of the ginseng fermentation process. There are two common selective media that can be used to isolate Bifidobacterial from yoghurt, namely, De Man, Rogosa and Sharpe (MRS) agar, and galactose agar. MRS agar is a selective medium developed primarily for the isolation and cultivation of lactic acid bacteria [11]. This medium is suitable for lactic acid bacteria as it includes sorbic acid and is acidic with a pH of approximately 5.7. The medium is fortified with L-cysteine-HCl to decrease the medium's oxidation-reduction potential, thus leading to an increase in *Bifidobacterium* growth [12]. In brief, a 100 µL volume of diluted yoghurt is spread on the prepared MRS agar dish and then incubated for 48 hours in anaerobic condition at 37°C. The obtained colonies are separated on the foundation of different colony morphology and sub-cultured on fresh MRS 0.05% L-cysteine Petri dishes to get the pure strains [13].

Galactose agar containing lithium chloride is proven to be a suitable medium for the aim of *Bifidobacterium*'s selective enumeration from commercially sold fermented dairy products. This medium offers marked advantage compared to other conventional media as it is highly selective towards Bifidobacteria. Consequently, antibiotics is not required to be added into the prepared medium to inhibit the growth of other bacteria existing in the multi-strain yoghurt culture. The obtained colonies after incubation can be identified on the foundation of different colony morphology and sub-cultured in order to obtain pure strains [14].

3. Identification of bacterial strain

It is important to separate the Bifidobacteria strain from the remaining species in the commercial yoghurt prior to its application in the fermentation process of Vietnamese ginseng. This step aids in the downstream processes such as fermentation control, troubleshoot, and optimization of conditions. There are two methods that have been commonly performed for the identification purpose including polymerase chain reaction

and then sequencing or using carbohydrate fermentation patterns.

Bifidobacteria can perform the transformation of various carbohydrates including oligosaccharides and polysaccharides. Different *Bifidobacterium* strains behave distinct carbohydrate-utilizing abilities which can be distinguished based on the chemical color and pH indicator of the fermentation batch, also known as fermentation patterns [14]. This identification approach is called profiling strain using fermentation patterns. The profiling can be performed using modified MRS broth supplemented with 20 different sugars. The inoculum is prepared and incubated anaerobically in the modified MRS broth for 24 hours. After the incubation period, results can be observed by a color change of the phenol red indicator from red to yellow which demonstrates the acid generation by carbohydrate fermentation [15]. The collected results will be then compared to the reported fermentation patterns of *Bifidobacterium* spp. [15]. Profiling using fermentation pattern has been considered a classic tool for the identification purpose. However, it has certain drawbacks in terms of protocol and results. The profiling procedure involves different steps requiring a wide range of chemicals and equipment.

Additionally, the obtained results encounter accusation for risk of misidentification and low accuracy [16]. In contrast to profiling bacterial strains using fermentation patterns, the polymerase chain reaction technique (PCR) offers a more convenient approach for the identification of microorganism owing to its high sensitivity, specificity, and efficiency. The identification of *Bifidobacterium* strains can be conducted using the Universal Method for bacterial detection and identification, which sequences the pure 16S rRNA gen and aligns it against the DNA database of bacteria. [17]. The 16S rRNA gene is a highly conserved component of the transcriptional machinery found in all bacteria. Thus, it can serve as a suitable target gene for DNA sequencing in samples comprising a wide range of species. The 16S rRNA gene also has conserved and variable regions, which makes it extremely beneficial for PCR mass scale identification. Sequencing the variable areas allows for the distinction between bacteria, archaea, and microbial eukarya while the conserved region allows for universal amplification [18]. The two universal primers known as 27 forward primer (27F) and 1492 reverse primer (1492R) are used to amplify the 16S rRNA gene. However, in order to generate short read sequences after the initial amplification of nearly full length bacterial 16S rRNA gene for rapid detection, the primer pair of 518F and 799R should be added [19]. The targeted DNA fragments are amplified along with the negative control and the positive control containing *E. coli* genomic DNA. It is suggested to utilize primers 518F/799R and cycle sequencing kit to create short read sequences from the purified products of the PCR phase in order to enhance the quality of the final results. An automated DNA sequencing system can be used to analyse the resultant sequences [20].

Table 1. Profiling differentiation of *Bifidobacterium* strains-based carbohydrate fermentation patterns. [15].

Strain	Substrate																			
	Arabinose	Cellobiose	Glycogen	Lactose	Lactulose	Maltose	Mannitol	Mannose	Melezitose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Starch	Sucrose	Tagatose	Trehalose	Xylose
B. adolescentis																				
ATCC 15703 (type)	-	-	-	+	+	+	-	-	-	+	+	-	+	-	+	-	+	-	-	-
ATCC 15704	-	-	-	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-
ATCC 15705	+	+	-	+	+	+	-	-	-	+	+	-	+	+	+	+	+	-	-	+
ATCC 15706	-	-	-	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-
B. angulatum																				
ATCC 27535 (type)	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-
B. bifidum																				
ATCC 11863	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 15696	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 29521 (type)	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. breve																				
ATCC 15698	-	-	+	+	+	+	-	+	-	+	+	-	+	-	-	-	+	-	-	-
ATCC 15700 (type)	-	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	-
ATCC 15701	-	+	-	+	+	+	+	+	-	+	+	-	+	-	+	-	+	-	-	-
B. catenulatum																				
ATCC 27539 (type)	-	-	-	+	+	+	-	-	-	+	+	-	+	-	+	-	+	-	-	-
B. infantis																				
ATCC 15697 (type)	-	-	-	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-
ATCC 15702	-	-	-	+	+	+	-	+	-	+	+	-	+	-	-	-	+	-	-	+
ATCC 17930	-	-	-	+	+	+	-	+	-	+	+	-	+	-	-	-	+	-	-	+
ATCC 25962	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+
ATCC 27920	+	-	-	+	+	+	-	+	-	+	+	-	+	-	-	-	+	-	-	-
B. longum																				
ATCC 15707 (type)	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+
ATCC 15708	+	-	-	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+
B. pseudocatenulatum																				
ATCC 27919 (type)	+	-	+	+	+	+	-	-	-	+	+	-	+	+	+	-	+	-	-	+
Variance (s^2)	0	0.37	0.32	0.03	0.05	0.03	0.11	0.05	0.05	0.05	0	0	0.16	0.11	0.11	0.11	0.11	0	-	0.05

Each strain was tested in duplicate

Table 2. Major differences in fermentation pattern of *Bifidobacterium* spp. found in human body [15].

Substrate				**Fermentation pattern**	
				Macromethod	Micromethod
Arabinose -	Ribose -			*B. bifidum* ATCC 29521	*B. bifidum* ATCC 29521, 11863, 15696 *B. infantis* ATCC 25962
	Ribose +	Mannose -	Cellobiose -	*B. infantis* ATCC 15697	*B. infantis* ATCC 15697 *B. adolescentis* ATCC 15703, 15704, 15706 *B. catenulatum* ATCC 27539
		Mannose +	Cellobiose -		*B. infantis* ATCC 15702, 17930 *B. breve* ATCC 15698
			Cellobiose +	*B. breve* ATCC 15700	*B. breve* ATCC 15700, 15701
Arabinose +	Salicin -	Melezitose -		*B. infantis longum*	*B. infantis* ATCC 27920 *B. angulatum* ATCC 27535
		Melezitose +		*B. longum* ATCC 15707	*B. longum* ATCC 15707, 15708
	Salicin +	Mannose -	Ribose -	*B. catenulatum* ATCC 27539 *B. angulatum* ATCC 27535	
			Ribose +	*B. adolescentis* ATCC 15703	*B. adolescentis* ATCC 15705 *B. pseudocatenulatum* ATCC 27919
		Mannose +		*B. pseudocatenulatum* ATCC 27919	

4. Biotransformation of ginsenosides from Ngoc Linh ginseng extract with *Bifidobacterium*

Performing biotransformation on ginseng extract can improve the pharmacological activities of the bioactive compounds through hydrolysis. *Bifidobacterium* spp. are suitable species for this role as they are commercially available and exhibit a high β-glucosidase activity. Fermentation of ginseng using Bifidobacteria is an efficient approach to convert majonosides to minor ginsenosides like Rh1 and F2 by detaching the sugar residues from the initial structures [20].

Prior to the fermentation, the ginseng undergoes the extraction process starting with grinding the dried ginsengs into fine powder. Reflux process is carried out in the ratio of 40g ginseng powder and 500 mL methanol-grade analytical reagent for 4 hours for each batch. The mixture is then vacuum filtered before dissolving in distilled water. Different adsorbent materials can be applied for additional purification with constant distilled water rinsing. The obtained crude continues to be suspended in absolute ethanol. The residue obtained after ethanol removal is dissolved in distilled water again to be the final ginseng extract [21]. The fermentation process can be conducted by using either growing cells or non-growing cells. However, the biotransformation using growing cells is associated with a number of drawbacks. The first disadvantage is that the growing process of microorganism requires a significant amount of metabolic energy that could have been otherwise diverted into the desired conversion. Secondly, the utilization of growing cells in the fermentation process may display numerous alternative pathways leading to low conversion rate. In attempt to tackle this issue, non-growing cells can be used. Non-growing cells can be simply prepared by resuspending the active cells in a nitrogen-deficient buffer. Non-growing cells offer a range of benefits for both the fermentation of ginseng and the downstream assessment. This technique does not require the diversion of precursors and metabolic energy to initiate the biotransformation, thus, inducing a marked volumetric productivity. Moreover, the resting cells also introduce a great opportunity of reusing the developed microorganism for the subsequent batches.

Conversely, using non-growing cells in biotransformation may retain some disadvantageous properties. Since these cells are not completely developed, they may result in an uncertain stability of the cell-origin enzyme and the cell's half-life which are the two factors that directly affect the fermentation efficiency. As previously mentioned, the cell growth is restrained leading to an issue in which certain substrate transport may be slowed down and the conversion of substrates to products may inhibit. Hence, the utilization of non-growing cells in fermentation process of ginseng is less prominent [6, 22]. In addition to fermentation of primary ginseng extract, several studies have been focused on fermenting the protopanaxadiol type ginsenosides. Before being able to perform the fermentation of protopanaxadiol type ginsenosides, it is required to isolate these ginsenosides from the crude extract. This purpose can be accomplished by using a methanol-conditioned separation cartridge to remove carbohydrates and impurities from diluted ginseng extract. The obtained product is eluted in three fractions using methanol solution of three concentrations, 48%, 71% and 100%. Ginsenosides of the protopanaxadiol type are found in the second fraction produced from the elution of 71% methanol, which is subsequently concentrated under decreased pressure before being dissolved in distilled water and lyophilized [21]. The fermentation of

the lyophilized protopanaxadiol ginsenoside extract is conducted using non-growing cells. The fermentation batch is incubated and monitored at different time-points. In order to stop the fermentation process once the fermentation time-points are achieved, the fermented samples and controls are heated for 30 minutes at 60°C [21].

5. Assessment of *in vitro* biotransformation rate

It is challenging to accurately and precisely estimate the microbial biotransformation rate as the kinetic models for a large mixture of compounds and enzymes are highly complexed and have not yet been officially established [23]. A general principle for basic assessment of the fermentation process is by observing the decrease in concentration of the majonoside in the initial sample, and the appearance or increase in concentration of a novel ginsenoside in the final mixture after the biostransformation. These changes in composition can be conveniently detected by different methods, two of which are TLC and HPLC.

TLC offers an accessible technique to initially reveal the formation of products [24]. To ascertain the ginsenoside metabolite compositions in the acquired fermented samples and controls that are removed at each stage of the fermentation process, HPCL can be used. Standards Rb1, Rc, Rb2, and Rd, as well as PD aglycone should be obtained from commercial sources for construction of standard curves for the quantification of ginsenoside metabolites. Calculation of the ginsenosides in all samples can be performed by applying the linear regression equations of the standard curves. The peaks can be assigned from the comparison the samples' retention times with that of each reference compound and confirmed as per their m/z provided by the LC-MS data [21]. In addition to the two previously mentioned techniques, the β-glucosidase activity assay is conducted to estimate the biotransformation rate and evaluate the activity of the fermentation batch over time. The fermentation of ginsenoside using Bifidobacteria lactis exerted a higher activity compared to the sample using *Lactobacillus rhamnosus* in spite of the general increasing trend in β-glucosidase activty of both microorganisms [21].

6. Conclusion

Ngoc Linh ginseng has presented prominent potential of becoming Vietnam's highly valuable health product. This review is a close examination of published data and results acquired from various research projects on ginseng fermentation using lactic acid bacteria. It can be concluded that the general fermentation protocol is applicable for Vietnamese ginseng species. The proposed strategies for enhancing biotransformation of Vietnamese ginseng include optimizing the extraction process of fresh Vietnamese ginseng and the β-glucosidase activity of Bifidobacteria. Further research is suggested to confirm the mass production of novel bioactive ginsenosides using fermentation method.

Funding Information

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2018.326.

References

[1] Bahukhandi, A., S. Upadhyay, and K. Bisht. (2021). *Panax ginseng* ca Meyer, in Naturally Occurring Chemicals Against Alzheimer's Disease. Elsevier. p. 217-223.

[2] Duc, N.M., et al. (1994). Saponins from Vietnamese ginseng, *Panax vietnamensis* Ha et Grushv. collected in central Vietnam. III. Chemical & pharmaceutical bulletin. *42*(3). p. 634-640.

[3] Yamasaki, K. and O. Tanaka. (1994). Saponins from Vietnamese Ginseng, *Panax vietnamensis* HA et Grushv. collected in central Vietnam. II. Chemical and Pharmaceutical Bulletin. *42*(1). p. 115-122.

[4] Yu, S., et al. (2017). Microbial transformation of ginsenoside Rb1, Re and Rg1 and its contribution to the improved anti-inflammatory activity of ginseng. Scientific Reports. *7*(1). p. 138.

[5] Cui, L., et al. (2016). Microbial conversion of major ginsenosides in ginseng total saponins by *Platycodon grandiflorum* endophytes. Journal of Ginseng Research. *40(*4). p. 366-374.

[6] Kim, J.H., et al. (2019). Enhancing immunomodulatory function of red ginseng through fermentation using *Bifidobacterium* animalis subsp. lactis LT 19-2. Nutrients. *11*(7). p. 1481.

[7] O'Callaghan, A. and D. Van Sinderen. (2016). *Bifidobacteria* and their role as members of the human gut microbiota. Frontiers in microbiology. *7*. p. 925.

[8] Nah, S.-Y. (2014). Ginseng ginsenoside pharmacology in the nervous system: involvement in the regulation of ion channels and receptors. Frontiers in physiology. *5*. p. 98.

[9] Van de Casteele, S., et al. (2006). Evaluation of culture media for selective enumeration of probiotic strains of lactobacilli and *Bifidobacteria* in combination with yoghurt or cheese starters. International Dairy Journal. *16(*12). p. 1470-1476.

[10] Arboleya, S., et al. (2016). Gut *Bifidobacteria* populations in human health and aging. Frontiers in microbiology. *7*. p. 1204.

[11] Renschler, M.A., et al. (2020). Using nitrous acid-modified de Man, Rogosa, and Sharpe medium to selectively isolate and culture lactic acid bacteria from dairy foods. Journal of dairy science. *103*(2). p. 1215-1222.

[12] Chowdhury, T. and J. Ferdouse. (2012). Isolation, characterization and antimicrobial activity of lactic acid bacteria from local milk and milk products. Bangladesh Journal of Microbiology. *29*(2). p. 76-82.

[13] Chowdhury, S., M.S. Ferdous, and J.M. Jose. (2016). A user-study examining visualization of lifelogs. in 2016 14th International Workshop on Content-Based Multimedia Indexing (CBMI).

[14] Iwana, H., et al. (1993). Isolation and identification of *Bifidobacterium* spp. in commercial yogurts sold in Europe. *Bifidobacteria* and Microflora. *12*(1): p. 39-45.

[15] Roy, D. and P. Ward. (1990). Evaluation of rapid methods for differentiation of *Bifidobacterium* species. Journal of Applied bacteriology. *69*(5). p. 739-749.

[16] Ventura, M., V. Meylan, and R. Zink. (2003). Identification and tracing of *Bifidobacterium* species by use of enterobacterial repetitive intergenic consensus sequences. Applied and Environmental Microbiology. *69*(7). p. 4296-4301.

[17] Barghouthi, S.A. (2011). A universal method for the identification of bacteria based on general PCR primers. Indian journal of microbiology. *51*. p. 430-444.

[18] Caporaso, J.G., et al. (2011), Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the national academy of sciences. *108*(supplement_1). p. 4516-4522.

[19] Ghyselinck, J., et al. (2013). The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. PloS one. *8*(8). p. e71360.

[20] Kim, E.-R., et al. (2010). Comparison of Bifidobacteria selective media for the detection of Bifidobacteria in Korean commercial fermented milk products. Korean J Food Sci Animal Res. *30*. p. 154-162.

[21] Tan, J.S., C.-R. Yeo, and D.G. Popovich. (2017). Fermentation of protopanaxadiol type ginsenosides (PD) with probiotic *Bifidobacterium* lactis and *Lactobacillus rhamnosus*. Applied microbiology and biotechnology. *101*. p. 5427-5437.

[22] Ng, Wenfa. (2020), Metabolism of non-growing cells and their application in biotransformation.Preprints. doi:10.20944/preprints202007.0402.v1

[23] Kebamo, S., S. Tesema, and B. Geleta. (2015). The role of biotransformation in drug discovery and development. J Drug Metab Toxicol. *6*(196). p. 2.

[24] Kang, B., et al. (2013). Ginsenoside derivatives and quality characteristics of fermented ginseng using lactic acid bacteria. Korean Soc Food Preserv. *20*. p.573-82.