Original Article

Development and Standardization of a Probiotic-Enriched supplement for CKD

Kowlini Mohanasundaram[1], Kalaivani Devarajan[2], Hemamalini Arambakkam Janardhanam[1,*]

[1]Department of Clinical Nutrition, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, Tamil Nadu, INDIA.
[2]Department of Biotechnology, Faculty of Biomedical Sciences and Technology, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, Tamil Nadu, INDIA.

Submission Date: 15-06-2021; Revision Date: 16-07-2021; Accepted Date: 20-08-2021

ABSTRACT

Introduction: Probiotics are suggested to be potentially therapeutic in the treatment and management of chronic kidney disease (CKD), as they improve gut microbiota, and delay the progression of CKD. Synbiotic benefits are found in *Lactobacillus acidophilus and Streptococcus thermophiles,* enriched with barely medium (prebiotic), in reducing Blood Urea Nitrogen, Uric acid and creatinine level. Therefore, this study aimed to standardize and develop a probiotic-enriched dietary supplement with barley as the prebiotic base in powdered form, and to prevent uremic toxins by targeting bacterial protein fermentation in gut. **Materials and Methods:** Half of each vial containing *L. acidophilus (*MTCC447) and *S. thermophilus (*MTCC1938) were mixed in 4ml sterile Luria Broth separately and incubated overnight. One ml of each mother culture was transferred to 4ml of selective medium (Nutrient broth) separately and kept for overnight incubation. These two probiotic microorganisms were transferred to prebiotic (enrichment) medium (barley). The colony forming units (CFU) in the enriched nutrient agar medium were counted. The serial dilutions $10^{-3}$and 10^{-6} were selected from the serially diluted culture. The scaled-up microorganisms *L. acidophilus* and *S. thermophilus* in the enriched medium were lyophilized. After lyophilisation, cultures were revived and checked for contamination by Gram staining method. CFU were counted to determine the scale-up of the microbes in the enriched medium. **Results:** There was a significant scale up of the probiotic in the barely medium in the study. **Conclusion:** Probiotics in barley medium can be used in the management and treatment of CKD, however future studies are required to confirm these findings.

Key words: Probiotic, *Lactobacillus acidophilu, Streptococcus thermophilus*, symbiotic, CKD, Colony forming units

Correspondence:
Dr. Hemamalini Arambakkam Janardhanam,
Department of Clinical Nutrition, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai-600 116, Tamil Nadu, INDIA.
Phone no: +91 9841327708

Email: hemajanardh@gmail.com

INTRODUCTION

Chronic kidney disease (CKD), marked by a progressive deterioration of kidney function overtime,[1] is a worldwide public health concern[2] with a globally increasing prevalence of 29.3% since 1990.[3] CKD significantly contributes to the global health burden as a cause of mortality and morbidity, and risk factor for cardiovascular disease (CVD).[3] The condition progresses from stages 1-5, as very mild damage to complete kidney failure, respectively.[4] The progression of CKD is related to health complications such as hyperlipidaemia, metabolic bone disease, anaemia, and CVD,[5] emphasising the importance of early detection. However, in the initial stages, CKD commonly occurs asymptomatically, with this also being when interventions appear to be most effective in the prevention or delay of its progression.[6,7]

One such possible intervention demonstrating effectiveness in the delaying of CKD is probiotic supplementation.[8] Probiotics are living bacteria and yeasts that yield beneficial effects on the body.[9] Researchers have suggested probiotic supplementation to possibly delay CKD progression by altering and

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2021.10.67

controlling intestinal flora, thus decreasing the harmful toxin, urea.[10] Dysbiotic gut microbiota demonstrates a relationship with CKD,[11] furthering the importance of probiotic supplementation in the initial stages. Moreover, probiotics improve intestinal barrier function,[12] modulate immune function,[13] and have the potential to fight diseases such as CKD.[14] Among stage 3 and 4 CKD patients, the oral ingestion of probiotics (90 billion colony forming units [CFUs]/day) was reported to be well tolerated and safe for humans, with a reduction in BUN, creatinine and uric acid levels.[15] For a probiotic product to have potential health properties, a minimum count of 10^6–10^7 CFU/g probiotics has been suggested.[16] Despite their benefits, probiotics are expensive,[17] mainly due to being available in synthetic form.

Specifically, the combination of two probiotic species, *Lactic acid acidophilus* and *Streptococcus thermophiles,* have been reported to have potential therapeutic effects by decreasing concentrations of nitrogen containing metabolites, thus helping to maintain healthy kidney function.[14] Along with probiotics, the administration of prebiotics illustrates synbiotics.[18] Prebiotics are present in the form of short-chain carbohydrates that selectively expediting the growth of beneficial bacteria in the colon.[19] Barley acts as a prebiotic as it stimulates the growth of beneficial bacteria in the large intestine. By supplementing patients with a symbiotic of bacteria (probiotics) and beneficial fibre to facilitate the growth of beneficial bacteria (prebiotics), gut flora and health can be improved, therefore decreasing the production of toxins.

Considering the findings of existing literature regarding the benefit of probiotics, and specifically *Lactobacillus acidophilus and Streptococcus thermophiles* on CKD, the present study focuses on the development of a synbiotic complex product using the two probiotic species and barley as the prebiotic. Through this study, it is intended that this product can be used as an adjuvant therapy in the treatment of CKD, and delay its progression. Additionally, the present study seeks to prevent uremic toxins by targeting the process of bacterial protein fermentation in the gut.

METHODOLOGY

Bacterial strains and culture conditions

Bacterial Strains such as *Lactobacillus acidophilus* MTCC 447 and *Streptococcus thermophilus* MTCC 1938, were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) Institute of Microbial Technology, Shanti Path, 39A, Sector 39, Chandigarh, 160036.

Reviving the microbial cultures

Half of each vial containing the strains, *Lactobacillus acidophilus* MTCC447 and *Streptococcus thermophilus* MTCC 1938 were mixed in 4ml of sterile Luria Broth separately and incubated overnight. This was used as the mother culture. Then, 1ml of each mother culture was transferred to 4ml of selective medium (MTCC447 and MTCC1938) separately and kept for overnight incubation.

Preparation of the MTCC 447 Selective Medium for *Lactobacillus acidophilus*

The selective medium was prepared as per the instruction given in the MTCC literature. The selective medium i.e., 50ml of tomato juice was prepared and filtered. It was left overnight at 10°C. The pH was adjusted to 7. Skimmed milk powder (50gms) and yeast extract (2.5g) was added to it. The medium was made up to 500ml with distilled water and kept in autoclave for sterilization. *Lactobacillus acidophilus* MTCC 447 was cultured in the prepared selective medium and incubated at 37°C for 24 hr.

Preparation of the MTCC 1938 Selective Medium for *Streptococcus thermophiles*

The selective medium was prepared according to instructions from MTCC literature. The selective medium (MTCC 1938) 1g of yeast extract, 0.5g of beef extract, 2.5g of peptone and 2.5g of NaCl were taken and dissolved in distilled water. 7.5g of agar was added to it and the volume was made up to 500ml with distilled water. The prepared medium was kept in autoclave for sterilization at 37°C for 24 hr. The pH was adjusted to 6.8 to 7 as per the instruction given in the MTCC literature. *S. thermophilus* MTCC1938 was cultured in the prepared selective medium and incubated at 37°C for 24 hr. For further use, the grown cultures (*L. acidophilus* MTCC447 and *S. thermophilus* MTCC1938) from the selective medium were streaked on Nutrient Agar petri plates.

Preparation of Cereal-based prebiotic medium as Enrichment Media for culturing Probiotic bacteria

Barley grain was used for the preparation of prebiotic media. The grain was ground in a laboratory mixer. The resulting flour weighed approximately 30 g. The flour was divided into two portions each weighing 15 g; and was added to two separate conical flasks containing 500 ml of nutrient agar medium each. After this, the medium was subjected to autoclaving at 121°C for

20 min for the purpose of sterilisation. The cereal-based prebiotic medium prepared was then cooled and used for growing the two probiotic cultures separately.

Enumeration of Colony Forming Unit

Principle

Microbial counting is a method used for determining the number of bacteria present for physiological or biochemical studies. The number of bacteria present in the unknown sample can be enumerated by making dilutions of the culture and counting the number of colonies formed upon overnight incubation of the diluted culture on agar plates. The individual CFU represents the bacterium present in the diluted culture. The CFU is calculated by dividing the product of the dilution factor and the volume of the diluted suspension which was plated. The number of bacteria per mL that is present in the original solution is thus calculated.

Preparation of Culture Media for Enumeration of Colony Forming Unit

Half of the strains of lactobacillus acidophilus and streptococcus thermophillus were mixed with 2ml of Luria broth medium separately in sterilized test tubes. The inoculum for pour plate was made by mixing 1ml of each strain with 4ml of barley medium separately. 20 numbers of sterile 15ml test tubes were filled with 4ml of Luria Broth each for serial dilution, and 20 numbers of sterile 90mm petri plates were also taken for finding the colony forming unit in each dilution. 250 ml of Nutrient Agar medium was prepared for pour plate method.

Serial Dilution of Probiotic Culture

The two strains were inoculated separately in 5ml of barley medium each and kept in the shaker over a period of 24 hr. After 24 hr the strains were used for serial dilution. 1ml of the strain was mixed in the first test tube which was 10^{-1} dilution in the Barley medium. Serial dilution was performed from the first test tube by taking 1 ml of the mixed culture to the second tube which contained 4ml of barley medium. The total volume of the second dilution 10^{-2} was then 5ml, and the same procedure was subsequently followed for the remaining dilutions up to 10^{-10} dilution. 1ml of the medium was discarded from the last test tube which was 10^{-10} dilution. The solution, therefore, has been diluted by a factor of $10(10^{-10})$. The final dilution ratio was calculated from the serial dilution. The entire procedure was done for the two strains and was used for determining the CFU.

Enumeration of Colony Forming Unit of Probiotic Culture

100µl culture from each serially diluted test tube was taken and transferred to petri plates for pour plating. The plates were labelled as 10^{-1}, 10^{-2},10^{-3}... 10^{-10} respectively. Pour plate method was carried out by mixing the inoculum with the medium and then allowing it to solidify. The plates were incubated at 37°C for 24 hr and the colonies grown were counted. The minimum number of colonies from 30 to 300 were and used for scaling up in the barley enriched medium.

The number of bacteria per mL of diluted sample was calculated by the following formula:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL)} \times \text{total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Scale up of Probiotic in Barley Enrichment Medium

The *L. acidophilus* culture (4×10^{-2} dilution) was selected on the basis of ease of counting since the culture would be scaled up upon adding it to the enrichment medium. The successive dilution was not taken as it had higher number of colonies and would be difficult to count after scaling up in the enrichment medium. Similarly, the *S. thermophilus* (3x 10^{-6} dilution) was selected for the same reason as above. The preferred dilutions of each bacteria of volume 2 ml were taken and transferred to the barley medium of volume 500 ml each. The medium was then kept in the shaker for overnight incubation. The barley medium was then lyophilized.

Lyophilization of Barley Enrichment Medium

Lyophilization extracts water from foods and other products to ensure foods remain stable and can be stored in an ambient room temperature condition. Lyophilization is carried out through sublimation.[20] Sublimation denotes the transition of a substance from a state of solid to vapour, without initially passing through an intermediate liquid phase. For water extraction from foods, the following are the steps of the process of lyophilization:

1. Freezing food for water in food to turn into ice
2. The ice will then be sublimated to turn into water vapour under a vacuum
3. The water vapour will then be drawn off
4. Following sublimination, foods will be freeze-dried and removed from machine.

The barley medium containing the *L. acidophilus* (MTCC 447) and *S. thermophilus* MTCC 1938) were lyophilized in CHRIST lyophilizer machine. The lyophilized material was further used for determining the number of colonies present in the enrichment medium.

Enumeration of colony forming unit of the probiotic in Barley Enrichment Medium

The lyophilized culture weighing 250 mg was taken and diluted in 6 ml of the LB broth. Serial dilution was performed by adding 1 ml of the lyophilized culture to 4 ml of LB broth in the first test tube and subsequent serial dilutions were made as previously done. The number of colonies obtained from the pour plate was counted.

RESULTS

Enumeration of Colony Forming Unit of Probiotic Cultures

The *Lactobacillus acidophilus* (MTTC 447) and *Streptococcus thermophillus* (MTCC 1938), colonies formed in nutrient agar plates. The results are shown in Figure 1a and 1b and Table 1. The *L. acidophilus* culture (4×10^{-2} dilution) with the colony count of 27 was selected on the basis of ease of counting since the culture would be scaled up upon adding it to the enrichment medium. The successive dilution was not taken as it had a higher number of colonies and would be difficult to count after scaling up in the enrichment medium. Similarly, the *S. thermophilus* (3x 10^{-6} dilution) with colony count of 27 was selected for the same reason as above. The following formula was used for calculating number of bacteria per mL of diluted sample:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL)} \times \text{total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Lactobacillus acidophilus: 6.75×103/ml and *Streptococcus thermophillus*: 9.0×106/ml

Yield of Lyophilization

Lactobacillus acidophilus yields forty-seven grams and *Streptococcus thermophillus* yields fifty-nine grams.

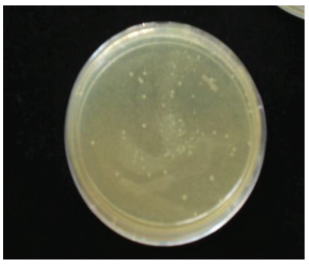


Figure 1a: Colony Forming Unit from Lactobacillus acidophilus (dilution 2×10-1).

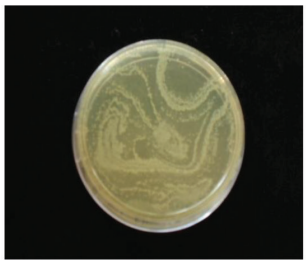


Figure: 1b: Colony Forming Unit from Streptococcuthermophillus(dilution2×10-1).

Figure 1: Nutrient agar plates showing Colony Forming Unit from culture (MTCC44, MTCC 1938).

Table 1: The number of bacteria per mL of serially diluted bacteria.

Dilution Factor	Number of bacterial colonies (CFUs)	
	L. acidophillus	*S. thermophillus*
2×10^{-1}	75	TNT (Too numerous to count)
4×10^{-2}	27	TNT
8×10^{-3}	14	TNT
1.6×10^{-4}	10	TNT
3.2×10^{-4}	17	86
6.4×10^{-6}	14	72
1.3×10^{-6}	12	36
3×10^{-6}	9	27
2.5×10^{-8}	6	18
5.1×10^{-8}	5	9

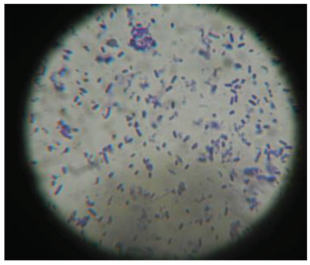


Figure 2a: Lactobacillus acidophilus gram positive bacteria stain deep violet to blue.

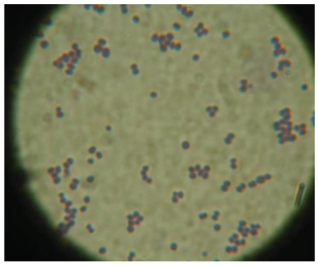


Figure 2b; Streptococcus thermophillus gram positive bacteria stain purple.

Figure 2: Identification of Probiotic Bacteria by Gram Staining.

Identification of probiotic strain from lyophilized culture by Gram's staining method

Rod shaped and purple-coloured organisms were considered Gram positive and cocci shaped and pink coloured organisms were considered to be Gram negative. The results are shown in Figure 2a and 2b. The lyophilized culture was serially diluted and the colonies formed in nutrient agar plates from various dilutions were counted. The results are shown in Figure 3a and 3b. The number of colonies was increased after lyophilization. The number of bacteria per mL of diluted sample was calculated, *Lactobacillus acidophilus*: TNTC (Too Numerous To Count)/ml, *Streptococcus thermophillus:* 57×10^{6}/ml.

DISCUSSION

The present study focused on the development and standardisation of a synbiotic complex using pre and probiotics. Barley medium was used as the prebiotic for the probiotics to grow. *Lactobacillus acidophilus* (MTCC 447) and *Streptococcus thermophillus (*MTCC 1938), were

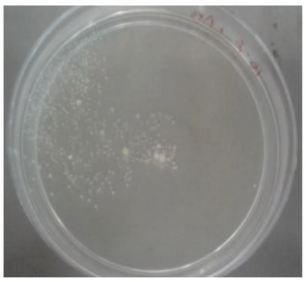


Figure 3a: Colony Forming Unit of Lactobacillus acidophilus (dilution 5.1×10-8).

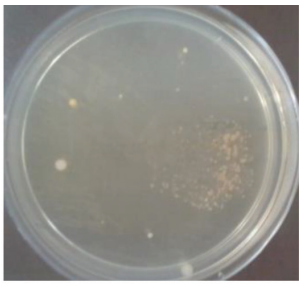
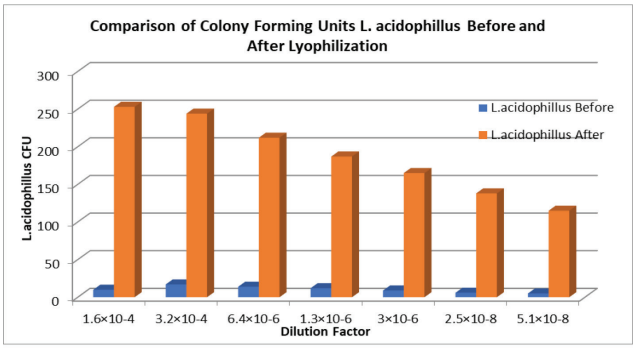


Figure 3b: Colony Forming Unit of Streptococcus thermophilus (dilution 5.1×10-8).

Figure 3: Colony Forming Unit of *Lactobacillus acidophilus and Streptocoocus thermophillus* in Nutrient agar plates.



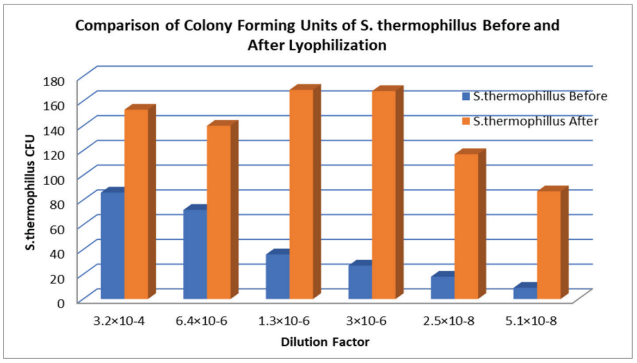


Figure 4: Graphical Representations of the Comparison of Colony Forming Units of *L. acidophillus* and *S. thermophillus* before and After Lyophilization.

obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) as the probiotic. These two probiotic microorganisms were used determining the colony forming unit in barely enriched prebiotic medium. The colonies were first formed in Nutrient agar medium. Two dilutions were selected on the basis of ease of counting since the culture would be scaled up upon adding it to the enrichment medium. The successive dilution was not taken as it had higher number of colonies and would be difficult to count after scaling up in the enrichment medium. The colonies formed in the selected dilutions were scaled up in the enrichment medium and was lyophilized. After lyophilisation, the cultures were stained by Gram staining method for checking the purity of the culture and the CFU was done to check the effectiveness of the enrichment medium in scaling up the probiotic culture. Finally probiotic microorganisms were scaled up when using the barley enriched medium. This dietary supplement was supplemented based on intervention needs. The product must be checked for shelf life and stability. Many researchers have reported the beneficial therapeutic effects of oral ingestion of Probiotics consisting an average of 90 billion colony forming units [CFUs]/day.[15,21] Figure 4.

A review on probiotic supplementation on CKD also suggests probiotics to decrease p-cresyl sulfate (PCS) and increase levels of interleukin-6, thus protecting the intestinal wall of individuals with CKD.[10] Although the decrease of urea and creatinine level is beneficial, the role of probiotics in preserving glomerular filtration rates (GFR) in CKD are yet to be studied and confirmed, thus demonstrating the ambiguity of results.[22] Another review posited that although there are insignificant alterations in serum creatinine of estimated-GFR following short-term probiotic treatment, there are potential beneficial impacts of probiotics on the presence of uremic toxins in individuals with CKD.[23] Among non-dialysis CKD individuals, probiotic supplementation exerted a positive influence on urea levels, while there was no evidence that probiotics decrease C-reactive protein, creatinine, eGFR, and uric acid.[24] Furthermore, synbiotic therapy was found to not reduce serum indoxyl sulfate, but reduced PCD levels and benefited the stool microbiome of patients.[25] Nonetheless, research has also asserted that the evidence on the use of prebiotics, probiotics, and synbiotics in the regulation of CKD is inconclusive.[26] Research has also shown *Faecalibacterium and Roseburia* to be less among early-stage kidney disease (ESKD) patients when compared to health individuals. This potentially indicates its contribution to gut dysbiosis. Also, Fusobacterium, Shewanella, and Erwinia were present in the ESKD patients, but absent in the health individuals. The study inferred that developing gut symbiosis may be an additional treatment for CKD patients once empirically strengthened.[24]

Despite the findings of these studies, healthcare providers are unable to firmly suggest probiotic products due to a lack of strong data. Apart from Renadyl by Kibow Biotech, commercial probiotics that focus on uremic toxics are also scarce.[28]

CONCLUSION

In the present study, the probiotics *Lactobacillus acidophilus* and *Streptococcus thermophillus* were found to be scaled up in prebiotic barley enriched medium and they were found to be viable after lyophilisation. This natural synbiotic product can be considered as a therapeutic adjunct to treat many disease conditions including CKD. To confirm these findings, future studies are needed to evaluate the short- and long-term effects of synbiotic complexes on the progressive CKD patients and their intestinal health.

ACKNOWLEDGEMENT

We gratefully acknowledge Department of Biotechnology, Sri Ramachandra Institute of Higher Education and research for helping in completion of this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CKD: Chronic Kidney Disease; **CFU:** Colony Forming Unit; **BUN:** Blood urea Nitrogen; **MTCC:** Microbial Type culture collection Gene Bank; **CVD:** Cardio Vascular Disease; ***L. acidophilus*:** Lactobacillus acidophilus; ***S. thermophilus:*** Streptococcus thermophiles; **PCS:** p-Cresyl Sulfate; **TNTC:** Too Numerous To Count; **GFR:** Glomerular filtration Rate; **eGFR:** Estimated Glomerular Filtration Rate; **ESKD:** Early -Stage Kidney Disease.

REFERENCES

1. Fraser SD, Blakeman T. Chronic kidney disease: identification and management in primary care. Pragmat Obs Res. 2016 Aug;7:21-32. doi: 10.2147/POR.S97310, PMID 27822135. Available from: /pmc/articles/PMC5087766.
2. Levey AS, Atkins R, Coresh J, Cohen EP, Collins AJ, Eckardt KU, Nahas ME, Jaber BL, Jadoul M, Levin A, Powe NR, Rossert J, Wheeler DC, Lameire N, Eknoyan G. Chronic kidney disease as a global public health problem: approaches and initiatives - A position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007 Aug;72(3):247-59. doi: 10.1038/sj.ki.5002343, PMID 17568785.
3. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2020 Feb 29;395(10225):709-33. doi: 10.1016/S0140-6736(20)30045-3, PMID 32061315.
4. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: A position statement from Kidney Disease: improving Global Outcomes (KDIGO). Kidney Int. 2005 Jun 1;67(6):2089-100. doi: 10.1111/j.1523-1755.2005.00365.x, PMID 15882252.
5. Thomas R, Kanso A, Sedor JR. Chronic kidney disease and its complications [internet]. Prim Care. 2008;35(2):329-44, vii. doi: 10.1016/j.pop.2008.01.008, PMID 18486718:[about 1p.]. Available from: /pmc/articles/PMC2474786.
6. Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Doi Y, Okubo K, Wakugawa Y, Hata J, Oishi Y, Shikata K, Yonemoto K, Hirakata H, Iida M. Chronic kidney disease and cardiovascular disease in a general Japanese population: the Hisayama Study. Kidney Int. 2005 Jul;68(1):228-36. doi: 10.1111/j.1523-1755.2005.00397.x, PMID 15954912.
7. Yuyun MF, Adler AI, Wareham NJ. What is the evidence that microalbuminuria is a predictor of cardiovascular disease events? [internet]. Curr Opin Nephrol Hypertens. 2005;14(3):271-6. doi: 10.1097/01.mnh.0000165895.90748.3b, PMID 15821422.
8. Dilip Kiran M, Gharat P, Vakharia M, Ranganathan N. Specific probiotics for chronic kidney disease: a review. Original article the Indian practitioner q. 2019.
9. RF. Probiotics in man and animals [internet]. Journal of Applied Bacteriology. Vol. 365(78). [p. about1p]; 1989. J Appl Bacteriol [cited Jun 3 2021]. Available from: https://pubmed.ncbi.nlm.nih.gov/2666378/.
10. Jia L, Jia Q, Yang J, Jia R, Zhang H. Efficacy of probiotics supplementation on chronic kidney disease: a systematic review and meta-analysis. Kidney Blood Press Res. 2018 Nov 1;43(5):1623-35. doi: 10.1159/000494677, PMID 30380555.
11. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, Desantis TZ, Ni Z, Nguyen TH, Andersen GL. Chronic kidney disease alters intestinal microbial flora. Kidney Int. 2013;83(2):308-15. doi: 10.1038/ki.2012.345, PMID 22992469.
12. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, et al. Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol. 2018 Aug 24;9(AUG):1953. doi: 10.3389/fmicb.2018.01953, PMID 30197632.
13. Yan F, Polk DB. Probiotics and immune health [internet]. Curr Opin Gastroenterol. 2011;27(6):496-501. doi: 10.1097/MOG.0b013e32834baa4d. Available from: /pmc/articles/PMC4006993.
14. Koppe L, Mafra D, Fouque D. Probiotics and chronic kidney disease [internet]. Kidney Int. 2015;88(5):958-66. doi: 10.1038/ki.2015.255, PMID 26376131.
15. Ranganathan N, Ranganathan P, Friedman EA, Joseph A, Delano B, Goldfarb DS, Tam P, Rao AV, Anteyi E, Musso CG. Pilot study of probiotic dietary supplementation for promoting healthy kidney function in patients with chronic kidney disease. Adv Ther. 2010 Sep;27(9):634-47. doi: 10.1007/s12325-010-0059-9, PMID 20721651.
16. Stanton C, Gardiner G, Meehan H, Collins K, Fitzgerald G, Lynch PB, Ross RP. Market potential for probiotics. Am J Clin Nutr. 2001;73(2);Suppl:476S-83S. doi: 10.1093/ajcn/73.2.476s, PMID 11157361.
17. Van Wagenberg CPA, van Horne PLM, van Asseldonk MAPM. Cost-effectiveness analysis of using probiotics, prebiotics, or Synbiotics to control Campylobacter in broilers. Poult Sci. 2020 Jun 10;99(8):4077-84. doi: 10.1016/j.psj.2020.05.003, PMID 32731995.
18. Vyas U, Ranganathan N. Probiotics, prebiotics, and Synbiotics: gut and beyond. Gastroenterol Res Pract. 2012;2012:872716/2012/87272716.Epub. doi:
19. Prasanna PHP, Rastall RA. Potential applications of prebiotics to yogurt and impact on health. In: Yogurt in health and disease prevention. Elsevier; 2017. p. 171-82.
20. Akers MJ, Fites AL, Robison RL. Formulation design and development of parenteral suspensions [internet]. J Parenter Sci Technol. 1987;41(3):88-96. PMID 3612415.
21. Ranganathan N, Ranganathan P, Vyas U, Friedman EA. Probiotics for chronic kidney disease: the "RENADYL". Story. 2014 Jul 24:1155/2014/568571. doi:
22. Lempert KD. Probiotics and CKD progression: are creatinine-based estimates of GFR applicable? [internet]. Am J Kidney Dis. 2019;74(4):429-31. doi: 10.1053/j.ajkd.2019.02.003, PMID 30910371.
23. Thongprayoon C, Hatch ST, Kaewput W, Sharma K, Ungprasert P, Wijarnpreecha K, D'Costa M, Mao MA, Cheungpasitporn W. The effects

of probiotics on renal function and uremic toxins in patients with chronic kidney disease; a meta-analysis of randomized controlled trials [internet]. J Nephropathol. 2018;7(3):106-14. doi: 10.15171/jnp.2018.25.

24. Tao S, Tao S, Cheng Y, Liu J, Ma L, Fu P. Effects of probiotic supplements on the progression of chronic kidney disease: A meta-analysis. Nephrology (Carlton). 2019 Nov 30;24(11):1122-30. doi: 10.1111/nep.13549, PMID 30561114.
25. Rossi M, Johnson DW, Morrison M, Pascoe EM, Coombes JS, Forbes JM, Szeto CC, McWhinney BC, Ungerer JP, Campbell KL. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): A randomized trial. Clin J Am Soc Nephrol. 2016 Feb 5;11(2):223-31. doi: 10.2215/CJN.05240515, PMID 26772193.
26. McFarlane C, Ramos CI, Johnson DW, Campbell KL. Prebiotic, probiotic, and synbiotic supplementation in chronic kidney disease: A systematic review and meta-analysis. J Ren Nutr. 2019 May 1;29(3):209-20. doi: 10.1053/j.jrn.2018.08.008, PMID 30366767.
27. Yang CY, Chen TW, Lu WL, Liang SS, Da Huang HD, Tseng CP, Tarng DC. Synbiotics alleviate the gut indole load and dysbiosis in chronic kidney disease. Cells. 2021 Jan 9;10(1):114. doi: 10.3390/cells10010114, PMID 33435396.
28. Zirker L. Probiotic use in chronic kidney disease patients [internet]. J Ren Nutr. 2014;24(6):e47-9. doi: 10.1053/j.jrn.2014.08.004.

Cite this article: Kowlini M, Kalaivani D, Hemamalini AJ. Development and Standardization of a Probiotic-Enriched Dietary Supplement. AJBLS. 2021;10(2):507-13.