Biological Forum
an International Journal 17(4): 60-64(2025)
ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Production of Folic Acid using Folate-producing Bacteria from Different Prebiotic Sources

Madhusmita Konwar[1], Pranit Saikia[2] and Jyotismita Konwar[3]*
[1]Project Associate, DBT-NER Advanced Level Biotech Hub Dibrugarh Hanumanbax Surajmall Kanoi College, Dibrugarh (Assam), India.
[2]Assistant Professor, Centre for Studies in Biotechnology and Bioinformatics, Dibrugarh University (Assam), India.
[3]Ph.D. Scholar, Food Science and Nutrition, College of Community Science, AAU-Jorhat (Assam), India.

(Corresponding author: Jyotismita Konwar)*
(Received: 30 January 2025; Revised: 06 March 2025; Accepted: 24 March 2025; Published online: 15 April 2025)
(Published by Research Trend)

ABSTRACT: The folic acid is known as vitamin B_9 which is converted into folate by body used as a dietary supplement and in food fortification as it is more stable during the processing and storage. Folic acids are used to treat or prevent folate deficiency anaemia, help baby's brain development, shull and spinal cord develop properly in pregnancy, prevent liver and heart disease, to avoid development problems such as spina bifida etc. The source of folate is dark green leafy, vegetables, beans, peas, nuts, fermented vegetables, probiotic tablet, orange, lemon, bananas etc. The fermented product contains probiotic source which can produce folate. The study was conducted by using prebiotic sources like curd, cheese and Bifilac tablet. The microorganism isolated from prebiotic source could use as probiotics in controlling the harmfull microorganism and restoring the natural microflora inside the gastrointestinal tract due to folate production. Thus, the presence of the health beneficial microflora will give a positive impact when consume pathogenic microbes. PABA (Para amino benzoic acid) is necessary for the synthesis of folic acid. When the Para amino benzoic acid was supplemented in the peptone broth the bacteria strains can produce folic acid.

Keywords: pre-biotic, probiotic, lactic acid bacteria, PABA, folic acid.

INTRODUCTION

Folate is a water-soluble vitamin and includes endogenous food folate and its synthetic form, folic acid. In its naturally occurring form folate lacks stability in food storage and preparation (Liang, 2020). However folic acid is stable and used for supplements and food fortification. There are many critical cellular pathways dependent on folate as a one-carbon source, including DNA, RNA, and protein methylation, as well as DNA synthesis and maintenance (He and Li 2023). A number of genetic polymorphisms affect critical components of folate pathways and metabolism, and have been associated with an increased risk for NTDs. However, the exact mechanism(s) by which folic acid reduces the risk of NTDs is not known and remains an active area of research. The source of folate is dark green leafy, vegetables, beans, peas, nuts, fermented vegetables, probiotic tablet, orange, lemon, bananas etc. The fermented product contains probiotic source which can produce folate (Divya, 2016). The study was conducted by using prebiotic sources like curd, cheese and Bifilac tablet. the microorganism isolated from prebiotic source could use as probiotics in controlling the harmful microorganism and restoring the natural microflora inside the gastrointestinal tract due to folate production. Thus, the presence of the health beneficial microflora will give a positive impact when consume pathogenic microbes. PABA (Para amino benzoic acid) is necessary for the synthesis of folic acid. When the Para amino benzoic acid was supplemented in the peptone broth the bacteria strains can produce folic acid. As folate can produce nutrient which is essential for our body. But folate cannot be producing our body itself so it should take from outside of our body as pre-biotic source which can produce large number of probiotic bacteria in our body screening and quantification of folic acid produced by the microbial isolates.

The prebiotics concept was introduced for the first time in 1995 by Glenn Gibson and Marcel Roberfroid. Prebiotic was described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Davani-Davari *et al.,* 2019). Prebiotics are nondigestible dietary fibers that benefit the host health by stimulating the growth of probiotic microorganisms in the colon. Lactulose, galacto-oligosaccharides, fructo-oligosaccharides, xylo-oligosaccharide, malto-oligosaccharides, inulin, and its hydrolysates are some commonly used prebiotics comprising of two to ten sugar moieties. The end products of these prebiotics, i.e., acetate, butyrate, and propionate, act as energy sources for host organisms (Panesar *et al.,* 2014).

The following health benefits are attributed to prebiotics: relief from poor digestion of lactose, increased resistance to bacterial infection, better immune response and possible protection against cancer, reduction of the risk of diseases such as intestinal disease, cardiovascular disease, non-insulin dependent diabetes, obesity and osteoporosis (Valdemiro Carlos, 2011).

The term 'probiotic' is derived from Greek word 'pro' means 'life' and has several meanings over the years (Kodi *et al*., 2015). Probiotics are live nonpathogenic microorganism administered to improve microbial balance, particularly in the gastrointestinal tract (Williams, 2010). The term "probiotic" was first used in 1965, by Lilly and Stillwell, to describe substances secreted by one organism which stimulate the growth of another (Gupta and Garg 2009). Various bacterial genera most commonly consist of Lactic acid bacteria are *Lactobacillus, Bifidobacterium, Escherichia, Enterococcus, Streptococcus, lactococcus, Bacillus.* Sp (Gupta and Garg 2009). Some yeast sp. Like *Saccharomyces* spp and mold like *Aspergillus* spp. The ability of probiotics to enhance the nutritional content and bioavailability of nutrients and the scientific evidence for the usefulness of probiotics in alleviating the symptoms of lactose intolerance and in enhancing growth development is examined. The probiotics have been incorporated in various products, mainly fermented dairy foods (Kechagia *et al.,* 2013). Probiotics were originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota (Nagpal *et al*., 2012). Probiotics are potentially boosting the immune system and help intreating conditions like a, lactose intolerance, diarrhea, colitis, hypertension, cancer, constipation, food allergies, inflammation bowel diseases etc (Kodi *et al*., 2015). The key mechanism of probiotics is it can protect the host against intestinal disease include production of inhibitory substance, blocking of adhesion sites degradation of toxic receptor. Competition for nutrients with pathogenic bacteria, Immune modulation of nonspecific host resistance, Effect on stimulation of systemic immunity (Kodi *et al.,* 2015). Probiotic bacteria have multiple and diverse influences on the host. Different organisms can influence the intestinal luminal environment, epithelial and mucosal barrier function, and the mucosal immune system. They exert their effects on numerous cell types involved in the innate and adaptive immune responses, such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, including T cells with regulatory properties, and NK cells (Ng *et al.,* 2009).

Statement of Problem: In this study, several key issues were addressed regarding the use of folate-producing bacteria isolated from prebiotic sources. Given that folate is an essential nutrient that the human body cannot synthesize, its intake from external sources is critical. This research also aimed to investigate whether prebiotic sources could effectively promote the growth of probiotic bacteria capable of producing folate within the human body. Finally, the potential of these bacterial strains to prevent diseases associated with folate deficiency was also evaluated. Overall, this project seeks to establish the therapeutic significance of folate-producing probiotic bacteria as a natural means to enhance maternal and fetal health.

MATERIAL AND METHODS

The study was carried out using curd samples collected from local areas in the Sivasagar and Dibrugarh districts of Assam, cheese samples obtained from markets in the same districts, and the probiotic bacterium *Bifilac*, which was procured from a pharmacy in the Dibrugarh district of Assam.

1. Methodology. Serial dilution method was followed for preparation of Sample. Media preparation by weighted 5.515 gm of MRS agar and then mixed with 100ml distilled water and then heat for a clear solution. Plating of sample on MRS agar in spread plate method (Sathiah and Pan 2023). In order to check any contamination, one control plate was taken and other plates were sealed properly with parafilm taken for bacterial growth incubated at 35°C for 24 hours. Sub-culture of the microbes on MRS agar in streak plate method to get pure culture incubated it 35°C for 24hrs. Screening the isolated microbes by Total colony count. Morphological characterization was done by simple straining and gram staining method. then observed the slide under the compound microscope. A magnification of 40X to 100X is used to distinguished cell shape and arrangement. biochemical test like catalase test, Motility Test, Gas Production from glucose test were done. The isolated bacterial strains were inoculated in Lactose pepton broth with supplemented 1% PABA for 100ml media and another one was without PABA incubated in at 35°C for 24 hours. Measure the optical density under colorimeter.

2. Plating the serial diluted sample to MRS agar by spread plate methods:

RESULT

1. Isolation of bacteria from the samples: Serial dilution was done to estimate the concentration of samples from 10^{-1} to 10^{-5} to MRS agar. The CFUs generated from these dilutes were further investigated for morphological and biochemical characters. The isolated bacterial strains were inoculated in Lactose pepton broth with supplemented 1% PABA for 100ml media and another one was without PABA (Suwannaphan, 2021).

Table 1: Colony characterization on the basis of serial dilution.

Sample	Dilution	Color	Size	Morphology	Total CFUs count
Local curd-C1	10^{-4}	White=112	Small = 95	Circular=21	448
			Large= 17	Irregular=91	
Sweet curd	10^{-5}	White=134	Small=92	Circular=102	536
			Large=42	Irregular=32	

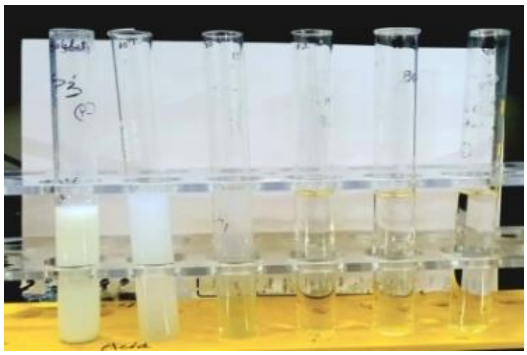

Fig. 1. Serial dilution using sample.

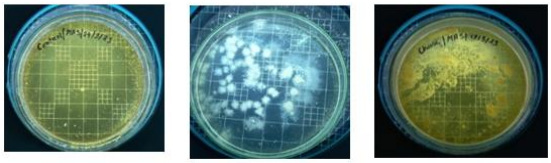

Fig. 2. CFUs developed from serial dilution in MRS agar after incubating at 35°C (48hrs).

Simple staining and gram staining method then observed the slide under the compound microscope. A magnification of 40X to 100X is used to distinguished cell shape and arrangement. biochemical test like catalase test, Motility Test, Gas Production from glucose test were done.

In above table1 total number of colony based on their color was calculated under colony counter. Comparing the morphology of the bacterial colonies such as size, color, different bacterial isolates were obtained which were further sub cultured and screened for further study.

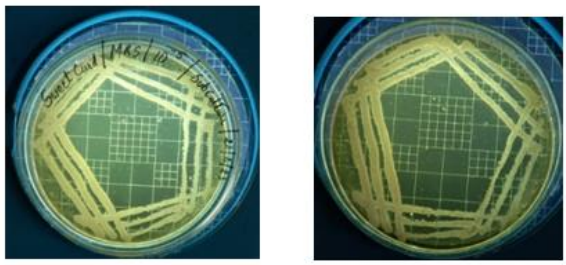

Fig. 3. Pure culture of targeted bacterial isolates.

In MRS broth the desired bacterial are suspended. This allows to grow up large amounts of bacteria for different types of biochemical test.

Fig. 4. All selected microbial strains are grown in MRS broth.

3. Morphoogical Characterization: Morphological features of isolates were determined by simple staining and gram staining.

(a) Simple Staining: During simple staining bacterial isolates, it can be concluded that each of them were gram positive bacteria.

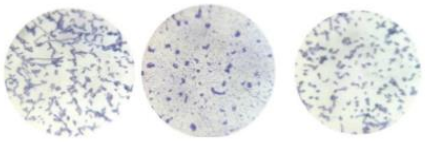

Fig. 5. Microscopic view of each selected microbial strains by using simple staining method.

Table 2: Result obtained after simple staining of the selected microbial isolates.

Microbial Strains	Gram Positive	Gram negative	Morphology	Shape
1. FA1	+	–	Bacilli	Rod
2. FA2	+	–	Cocci	Spherical
3. FA3	+	–	Bacilli	Rod

(b) Gram Staining: During Gram's staining of the microbial isolates, it can be concluded that each of them were Gram positive.

Table 3: Result obtained after gram staining of the selected microbial isolates.

Microbial Strains	Gram Positive	Gram negative	Morphology	Shape
1. FA1	+	–	Bacilli	Rod
2. FA2	+	–	Cocci	Spherical
3. FA3	+	–	Bacilli	Rod

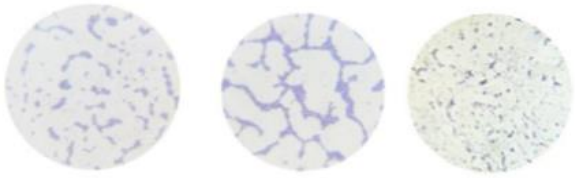

Fig. 6. Microscopic view of each selected microbial strains by using gram staining method.

Biochemical Characterization:

(a) Catalase test:

Table 4: Result obtained after subjecting the selected microbial isolates to Catalase test.

Sr. No	Biochemical tests	Microbial isolates		
		FA1	FA2	FA3
1.	Catalase test	-	-	-

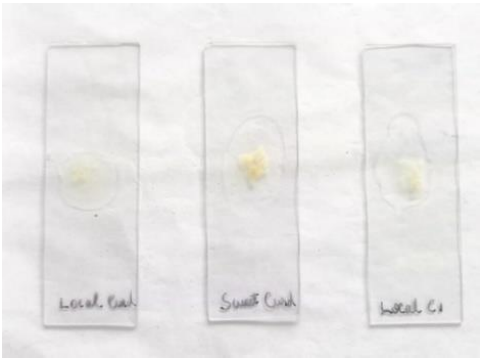

Fig. 7. Catalase test of selected microbial isolates.

From the Table 4 and observing Fig. 7 it can be concluded that the selected microbial isolates were catalase negative ; not formation of bubble that it was not able to produce the enzyme catalase & convert hydrogen peroxide into water and oxygen (Kaktcham *et al.*, 2012)

(b) Motility test:

Table 5: Result obtained after subjecting the selected microbial isolates to Motility test.

Sr. No.	Biochemical tests	Microbial isolates		
		FA1	FA2	FA3
1.	Motility test	+	+	+

Fig. 8. Motility test of selected microbial isolates.

Folate production:

Table 6: Result obtained after subjecting the selected microbial isolates to folate production observed in colorimeter.

	control	FA1	FA2	FA3
OD with PABA	0.149	0.703	1.639	0.712

	control	FA1	FA2	FA3
OD without PABA	1.240	1.424	2.000	1.313

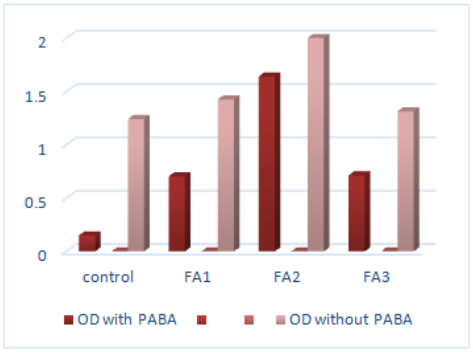

Fig. 9. Graph showing the growth of selected microbes in lactose peptone broth supplemented with PABA or without PABA.

From Table 6, bacterial strain FA2 shows highest growth with compare to the others in lactose peptone broth supplemented with PABA or without PABA that was also supported by Sybesma *et al.* (2003).

CONCLUSIONS

The folic acid is known as vitamin B_9 which is converted into folate by body is used as a dietary supplement and in food fortification as it is more stable during the processing and storage. Folate helps the body make healthy red blood cells and is found in certain foods. Folic acids are used to treat or prevent folate deficiency anaemia, help baby's brain development, shull and spinal cord develop properly in pregnancy, prevent liver and heart disease, to avoid development problems such as spina bifida etc. The source of folate is dark green leafy, vegetables, beans, peas, nuts, fermented vegetables, probiotic tablet, orange, lemon, bananas etc.

The fermented product contains a probiotic source which can produce folate. The biochemical test like catalase test, motility, gas production test that indicates the bacterial isolates are Lactic acid producing bacteria. Microorganism isolated from prebiotic source could use as probiotics in controlling the harmful microorganism and restoring the natural microflora inside the gastrointestinal tract due to folate production. Thus, the presence of the health-beneficial microflora will give a positive impact when consuming pathogenic microbes, when the para-aminobenzoic acid was supplemented in the peptone broth, because PABA is necessary for the synthesis of folic acid (Rossi *et al.*, 2011). The bacterial strain FA2 shows the highest growth in Fig. 9 with compare to the others in lactose peptone broth supplemented with PABA or without PABA.

FUTURE SCOPE

Based on the analysis of the study titled "Production of folic acid using folate-producing bacteria from different prebiotic sources", the future scope of this research lies in its potential applications in both healthcare and the food industry. The findings demonstrate that specific bacterial strains, particularly FA2, isolated from prebiotic sources like curd, cheese, and probiotic tablets, have a significant ability to synthesize folic acid when supplemented with PABA. This opens avenues for developing natural, microbe-based supplements or functional foods aimed at addressing folate deficiency-related disorders such as neural tube defects, anemia, and cardiovascular diseases. Furthermore, the use of such probiotics could play a pivotal role in restoring gut microflora and enhancing gastrointestinal health. Future research could focus on optimizing fermentation conditions, scaling up folate production using these strains, and integrating them into fortified food products or therapeutic probiotic formulations. Additionally, genomic and metabolic profiling of the most efficient strains like FA2 could enhance understanding of folate biosynthesis and improve bioavailability in functional food systems.

Acknowledgement. The authors are thankful to The Centre for Biotechnology and Bioinformatics, Dibrugarh University for providing all the facilities and relevant literature to conduct the experiment.

Conflict of Interest. None.

REFERENCES

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J. and Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92.

Divya, J. B. (2016). *Folate production by probiotic lactic acid bacteria* (Doctoral dissertation, Biotechnology Division, National Institute for Interdisciplinary Science and Technology (CSIR), Thiruvananthapuram).

Gupta, V. and Garg, R. (2009). Probiotics. *Indian Journal of Medical Microbiology*, *27*(3), 202-209

He, Q. and Li, J. (2023). The evolution of folate supplementation–from one size for all to personalized, precision, poly-paths. *Journal of Translational Internal Medicine*, *11*(2), 128-137.

Kaktcham, P. M., Temgoua, J. B. and Zambou, F. N. (2012). Characterization of lactic acid bacteria from fermented foods and evaluation of their probiotic properties. *African Journal of Biotechnology*, *11*(87), 15360–15368

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N. and Fakiri, E. M. (2013). Health benefits of probiotics: a review. *International Scholarly Research Notices*, *2013*.

Kodi, C., Gothandam, K. M. and Prabakaran, G. (2015). Identification and characterization of folic acid-producing potential starter for curd fermentation. *International Journal of Current Microbiology and Applied Sciences*, *4*, 118-130.

Liang, L. (2020). Folates: stability and interaction with biological molecules. *Journal of Agriculture and Food Research*, *2*, 100039.

Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S. and Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS microbiology letters*, *334*(1), 1-15.

Ng, S. C., Hart, A. L., Kamm, M. A., Stagg, A. J. and Knight, S. C. (2009). Mechanisms of action of probiotics: recent advances. *Inflammatory bowel diseases*, *15*(2), 300-310

Panesar, P. S., Bali, V., Kumari, S., Babbar, N. and Oberoi, H. S. (2014). Prebiotics. *Biotransformation of Waste Biomass into High Value Biochemicals*, 237-259.

Rossi, M., Amaretti, A. and Raimondi, S. (2011). Folate production by probiotic bacteria. *Nutrients*, *3*(1), 118–134

Sathiah, S. and Pan, I. (2023). Isolation of probiotic Lactococcus from milk and milk powder by serial dilution spread plate method to study antimicrobial activity. In *AIP Conference Proceedings*, *2655*(1). AIP Publishing.

Suwannaphan, S. (2021). Isolation, identification and potential probiotic characterization of lactic acid bacteria from Thai traditional fermented food. *AIMS microbiology*, *7*(4), 431.

Sybesma, W., Starrenburg, M., Tijsseling, L., Hoefnagel, M. H. N. and Hugenholtz, J. (2003). Effects of cultivation conditions on folate production by lactic acid bacteria. *Applied and Environmental Microbiology*, *69*(8), 4542–4548.

Valdemiro Carlos, S. (2011). The importance of prebiotics in functional foods and clinical practice. *Food and Nutrition Sciences*, *2011*.

Williams, N. T. (2010). Probiotics. *American Journal of Health-System Pharmacy*, *67*(6), 449-458.

How to cite this article: Madhusmita Konwar, Pranit Saikia and Jyotismita Konwar (2025). Production of Folic Acid using Folate-producing Bacteria from Different Prebiotic Sources. *Biological Forum*, *17*(4): 60-64.