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Review Article

Review on Vitamin Producing and Probiotic Properties of *Lactobacillus*, Derived from Tribal Fermented Foods

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ABSTRACT

Though almost all natural food sources contain vitamins, vitamin deficiencies are still prevalent in the World. In India, both macro and micronutrient deficiency diseases are very common due to low consumption of nutritional foods, consumption of foods with low nutritive value, improper cooking methods, poor socio-economic conditions etc. Lactic acid bacteria (LAB) are the best choice after food stuffs to reduce the burden of nutrient deficiency diseases. Recently lactic acid bacteria have been hugely used in the food industry for fermentation due to its numerous functions and health benefits. Fermentation with probiotic lactic acid bacteria not only enhances the nutritive value and digestibility of the foods but also enhances vitamin concentration due to their vitamin producing capability. Certain strains of lactic acid bacteria can synthesize B-group vitamins such as folate, cobalamine, riboflavin etc. This review listed current papers showing the probiotic and vitamin-producing properties of tribal fermented foods derived from Lactic acid bacteria including Lactobacillus. This review expects that the probiotic vitamin producing Lactobacillus strains can be used as a starter culture for the fermentation process to prepare indigenous fermented foods and helps in preventing clinical and subclinical vitamin deficiencies among tribal community.

Keywords: *Tribal fermented food, Lactobacillus, Probiotic, Vitamin producing LAB.*

INTRODUCTION

The tribal population of is one of the indigenous populations. Tribal populations in every state across India differ due to their different traditions, cultures, lifestyles, food habits and environments. In India, it is very difficult to fulfil the nutritional and health gap between non-tribal and tribal populations.

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The main hurdles behind their poor health and nutritional status are associated with their socio-economical status, cultural practices, geographic location, poor accessibility and availability of agricultural technology. According to the census, 2011 8.6% of the total Indian population belongs to the tribal community, which is 10.43 crore in number. In 1961 tribal population in India was 30.1 million and in 2011 it increased to 104.3 million, which is more than three times higher than previous one (Statistical Profile of Scheduled Tribes in India, 2013). According to National Nutrition Monitoring Bureau, 14.5% of tribal children (0-12 months) showed severe underweight. 11% and 16.4% of the tribal children (0-12 months) recognized as severe wasting and severe stunting respectively (Diet & Nutritional Status of Tribal Population, 2009). Both Asian and global (WHO) body mass index (BMI) cutoff levels indicate that BMI less than 18.5 indicates chronic energy deficiency. As per the data from the ministry of tribal affairs statistics division (2013) GOI, 46.6% of tribal women and 41.3% men (age 15-49 years) were chronically energy deficient. Micronutrient deficiency among both men and women ST population is very high due to their poor dietary pattern, majorly deficient in milk, milk product, fruits with poor living standards. It was estimated that only 33.5% women and 41.8% of men used to consume milk or curd weekly and 72.6% of tribal women do not keep fruits in their daily diet (Statistical Profile of Scheduled Tribes in India, 2013). Out of all indigenous population around the World scheduled tribe (ST) population in India shows the highest rate of infant mortality rate (IMR). According to NFHS- 4(2015- 2016), the Infant mortality rate was 44.4. The mortality rate of children in the age between 1 to 4 years and under five (U-5) children were 13.4 and 57.2 per 1000 live births, respectively (Tribal health in India, 2021).

Tribal population mainly resides in remote locations and is the most vulnerable (in terms of health and nutritional conditions) and socially deprived in India. Without improving the life status of the tribal population, it is not at all possible to ensure the socio-economic transformation of India as a whole. To ensure and promotion of their good health status, food and nutrition security consumption of natural, locally available tribal food is one of the significant options. Traditional tribal foods are sustainable, cost effective, easily available as well as accessible and some time they don't have to purchase also (Ghosh-Jerath et al., 2015). It has negligible negative impact on health. Indigenous fermented foods are an essential part of the daily diet of the tribal communities across India. Every fermented food contains a huge number of microorganisms that increases the quality and quantity of the proteins, including essential amino acids, and fatty acids present in the food.

The fermentation process can reduce the toxic content or detoxify harmful compounds in the food stuff. Through fermentation, we can get huge flavors diversity, various texture and aroma in the foods. Raw and processed foods can be preserved for longer period by various fermentation methods like lactic acid fermentation, acetic acid fermentation, alkaline and alcoholic fermentations. Another advantage of fermentation is that it can cut cooking times, reducing fuel consumption. Lactic acid bacteria are able to produce various antimicrobial compounds (hydrogen peroxide, bacteriocins and other antimicrobial peptides), organic acids (lactic acid, acetic acid), carbon dioxide, and ethanol that increase the quality of the fermented foods as well as control spoilage and foodborne disease causing pathogens (Gitishree et al., 2016) (Ghosh-Jerath et al., 2021). Fermented foods contain the numerous number of lactic acid bacteria, and many of them have probiotic properties. Probiotics are "live microbes which, when administered in adequate amounts, confer a health benefit to the host". Commonly used probiotics are *Lactobacillus* and *Bifidobacterium*. Certain disbalance in the intestinal microbiota cause several adverse effects, such as several metabolic diseases, inflammatory bowel diseases, cancer, autoimmune diseases and many others. Probiotic bacteria help to reduce several pathogens or bad bacteria, and their harmful effects and maintain the balance of normal gut microbiota (Rossi et al., 2011).

B vitamins are very essential for the metabolism of living organisms. B vitamins use as a precursor to initiating enzymatic reactions and electron transport chains. Microorganisms can synthesize B vitamins for their own benefits, but humans cannot, so humans depend on dietary sources to fulfil the daily requirements. B vitamins are naturally present in various food groups but till now many nations are facing burden of vitamin deficiency diseases and hidden hunger among special populations. In tribal population, vitamin deficiency is very common due to their unavailability of foods, unhealthy dietary pattern, inappropriate cooking methods, insufficient food intake and many more. Vitamins are essential for the normal metabolic functions of living organisms. Vitamins are used as precursors of intracellular coenzymes to maintain normal metabolic functions. The human body can't synthesize all the vitamins obtained from diet. Though vitamins are adequately present in different foods but deficiencies are still prevalent. B group vitamins are easily destroyed by heat treatment, cooking, soaking and other processing techniques (Gitishree et al., 2016) (Ghosh-Jerath et al., 2021).

Lactic acid bacteria including *Lactobacillus* are a heterogeneous group of bacteria present abundantly in the foods and inside the animal gastrointestinal and urogenital tract. Apart from the probiotic effect, *Lactobacillus* can also produce some macronutrients, including vitamins, and non-nutritional compounds. To overcome both macro and micro situation lactic acid bacteria (LAB) can be a sustainable and cost-effective way to prevent this vitamin deficiency. To encounter the deficiencies of vitamins use of vitamin producing microbes like Lactobacillus is one of the highly effective ways. Vitamin producing Lactobacillus is cost-effective, natural and non-toxic alternative to elevate the amount of vitamin concentration in the foods without any harmful adverse effects (LeBlanc et al., 2011). Lactic acid bacteria are able to produce mainly water-soluble vitamins (folates, riboflavin, vitamin B12). The main focus of this review is to evaluate vitamin producing capacity of *Lactobacillus*, present in different tribal fermented foods which going to helpful for tribal food fortification or increasing the vitamin concentration/ bioavailability of the already existing vitamin within foods (Gitishree et al., 2016) (Ghosh-Jerath et al., 2021).

Probiotic properties of *Lactobacillus*:

Handa et al. (2016) conducted one study and isolated *Lactobacillus plantarum*F22 from chhang, a traditional fermented beverage and confirmed its probiotic potentiality and survival ability against bile salt, low gastric pH. It could able to destroy a broad spectrum of foodborne and spoilage inducing microbes. The isolate was identified by conventional and molecular techniques and tested for different probiotic properties. The 16S rRNA sequence confirmed its effectiveness as the cumulative probiotic score was near about 91.7% (Handa et al., 2016). Kumara et al. (2016) selected some lactic acid bacteria from fermented foods and beverages of Kinnaur to check their probiotic properties including low pH, bile-salt tolerance, proteolytic, and antimicrobial, haemolytic activity. They mentioned two lactic acid bacterial strains namely, L1, L5 and L10 that had high stability against pH upto 2.0 and bile salt. All isolated strains exhibited antimicrobial activity but none of them had haemolytic activity. Among all the isolated strains only L5 used for bacteriocins production due to its significant probiotic properties. They measured the molecular weight of the bacteriocin near about 10 kD and concluded that the lactic acid bacteria L5 could survive in the gastrointestinal environment and be suitable for biopreservation and probiotics production (Kumara et al., 2016). Kumari et al. (2016) isolated twenty lactic acid bacterial strains from traditional fermented foods and beverages of north-western Himalayas and used 16S rRNA gene sequencing for identification. In vitro study revealed that among all the lactic acid bacterial strains such as *Lactobacillus brevis, Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* only *Lactobacillus brevis*PLA2, *Lactobacillus paracasei*PLA8 and *Lactobacillus brevis*PLA16 showed significant probiotic potentiality than any other commercial probiotic strains. *Lactobacillus brevis*PLA2 had maximum probiotic properties and also able to attach to human colonic cell line (HT-29) (Kumari et al., 2016). Sharma et al. (2017) used three different Khorisa varieties, a traditional fermented bamboo shoot product of Assam for lactic acid bacteria isolation. They evaluated their antimicrobial activity on various foodborne pathogens like *Listeria monocytogenes* (ATCC3452), *Staphylococcus aureus* (ATTC2346), *L. innocua* (ATCC3154) and *Enterococcus cloacae* (ATCC 35030) . The study selected some *Lactobacillus* strains such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, *Lactobacillus collinoides* for bacteriocin purification. Bacteriocins produced by those lactobacillus strains were effective against all the foodborne pathogens (clear inhibition zones visible) and this antimicrobial activity of bacteriocin were remain constant event after 121 ºC temperature for 15 minutes (Sharma et al., 2017). Rapsang et al. (2011) selected some Lactic acid bacterial strains that were isolated from tungtap, a traditionally fermented fish food product. The study choose foodborne pathogens like *Bacillus cereus* MTCC 430, *Salmonella typhi* MTCC 733, *Escherichia coli* MTCC 118, *Bacillus licheniformis* MTCC 429, *Klebsiella pneumoniae* MTCC 109 to confirm the bacteriocinogenic activity of the selected lactic acid bacterial strains and observed clear inhibition zones. The study detected among all selected Lactic acid bacterial strains Lactobacillus pobuzihii had maximum bacteriocinogenic activity against indicator pathogenic microorganisms (Rapsang et al., 2011). Traditional indigenous fermented foods of Meghalaya were also analyzed to check its probiotic potentiality. Some of the isolated *Lactobacillus* survived in different concentrations of bile salts, intestinal juices, gastric juice and pH 2-3. Isolated strains also showed free radical scavenging activity and antibacterial activity. The study confirmed the high probiotic potentiality of the isolates and stated that those isolates could be used to develop different fermented foods (Das et al., 2020). One experimental study evaluated the probiotic characteristics of lactic acid bacteria from kimchi. Study isolated *Lactobacillus curvatus* KCCM 43119, *Leuconostoc mesenteroides* KCCM 43060, *Weissella cibaria* KCTC 3746, and *Weissella koreensis* KCCM 41517 showed their antibacterial efficacy against different foodborne pathogens by using their cell free supernatant. Isolated lactic acid bacteria also had an acidic environment and bile salt tolerating capacity, significant coaggregation with foodborne pathogens. In the antibiotic susceptibility test, all stains showed resistance to erythromycin, gentamicin, chloramphenicol, streptomycin, kanamycin. While only *Weissella cibaria* KCTC 3746, and *Weissella koreensis* KCCM 41517 are resistant towards vancomycin. The study confirmed kimchi-derived lactic acid bacteria's antagonistic and probiotic activity (Choi et al., 2018). For the first time, Borah et al. (2016) isolated and characterized some new probiotic strains from Assam based traditional fermented fish and meat products. Among the all strains study choose *Staphylococcus sp.* DBOCP6 for the potent probiotic property. This specific strain had antagonistic activity, auto-aggregation activity, bile salt and wide range of pH (1 to 10) tolerant activity. This strain can survive within the gastrointestinal tract and in high temperatures (100 °C) (Borah et al., 2016). Sambanduram et al. (2018) collected fermented fish (*Utonga-kupsu*) from Manipur and identified some strains such as *Staphylococcus piscifermentans*, *Staphylococcus condimenti*, *Staphylococcus carnosus* and unknown *Staphylococcus.* Study evaluated the probiotic properties of those isolated strains and confirmed the bile salt, gastric juice tolerance, and antimicrobial activity (Sambanduram et al., 2018). Tamang B and Tamang J (2009) used traditional foods such as ekung, eup, and herring from Arunachal Pradesh isolated some *Lactobacillus* including *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus casei*, *Lactobacillus fermentum, Lactococcus lactis, and Tetragenococcus halophilus*. Study proved the probiotic properties of identified *Lactobacillus* strains (Tamang, 2009).

Vitamin producing ability of *Lactobacillus*:

Hati et al. (2019) used traditional rice fermented products of Garo Hills, Meghalaya, India for *Lactobacillus* strains isolation. They further selected five *Lactobacillus* strains to observe their vitamins (vitamin B12, vitamin-B2 and folate) and short chain fatty acids content capacity. Study used microbiological assay to detect vitamins production potentiality followed by High Performance liquid chromatography method for quantitative analysis. Milk medium and vitamins free assay medium were used to confirm vitamin and SCFA production ability respectively. Five strains of Lactobacillus identified by using 16S rDNA technique which were *Lactobacillus fermentum* (KGL2), *Lactobacillus plantarum* (KGL3A), *Lactobacillus fermentum* (KGL4), *Lactobacillus rhamnosus* (RNS4), *Lactobacillus fermentum* (WTS4). This study showed that those five specific Lactobacillus strains could produce all vitamins such as vitamin- B12, B2, folate and SCFAs including acetate, butyrate, lactate. After 36 hours of incubation among five strains *Lactobacillus plantarum* and *Lactobacillus fermentum* produced the highest amount of vitamin- B2 (0.7μg/ml) and vitamin-B12 (0.05μg/ml), respectively. *Lactobacillus fermentum* showed the highest capacity to produce folate after 24 hours, that was (0.09μg/ml) (Hati et al., 2019). Laiño et al. (2019) conducted an in vitro study to check the folate production and fol gene expression of *Streptococcus macedonicus* CRL415 under various conditions. They suggested that at 42°C temperature and pH 6.0 maximize fol gene expression, folate production rate. *Streptococcus macedonicus* CRL415 showed stability against various different conditions of the gastrointestinal tract and this strain also had probiotic properties (Laiño et al., 2019). In this study Li et al. (2017) total five *Enterococci* were isolated from infant feces to check their ability of vitamin B12 production. In vitro study confirmed that *Enterococcus faecium*LZ86 produced adenosyl cobalamin (report based on reversed phase high-performance liquid chromatogram) and had maximum B12 production capacity which was near about 499.8μg/L). Study evaluated safety level, probiotic properties of *Enterococcus faecium*LZ86 and ensure its stability in hot and cold temperature, osmotic stresses, ethanol, gastric acid (pH 2.0 for 3 hours), and bile salts (0.3%) (Li et al., 2017). Li et al. (2017) selected 31 LABs; among them, 18 strains could grow in the vitamin B12-free medium. 16S rDNA sequencing revealed that out of 18 strains, 7 were *Lactobacillus*. This present study used *Lactobacillus reuteri* ZJ03 as a positive control. The study detected two particular strains of *Lactobacillus*: *Lactobacillus plantarum* LZ95 (adenosylcobalamin producer and strain isolated from infant feces) and CY2 (methylcobalamin producer and strain isolated from fresh milk) as high amount of extracellular B12 producer. Production capacity were approximately 98 μg/L and 60 μg/L. Extracellular B12 producing strains could tolerate low temperature, gastric acid (pH 2- 3), bile salts (0.3%), high ethanol concentration. *Lactobacillus plantarum* LZ95 was detected as a good probiotic (Li et al., 2017). Panda et al. (2017) isolated Lactic acid bacteria from preterm babies and experimented to detect their folate producing ability. They observed bacterial strain named IFM4 produced maximum amount of folate (35ng/ml) than other isolated strains. They identified by 16S rRNA gene sequencing that the strain is similar to *Lactobacillus rhamnosus*. HPLC chromatogram revealed that this specific *Lactobacillus rhamnosus* strain formed 5-methyl tetrahydrofolate. The study also suggested that this specific strain had probiotic characteristics. After the in vitro experiment, they incorporated this strain into ragi gruel for fermentation and confirmed its ability to improve the food's digestibility, functional and therapeutic properties (Panda et al., 2017). D'Aimmo et al. (2012) selected 19 strains of *bifidobacteria* and analyzed their folate producing capacity by using HPLC. The study concluded that most *bifidobacteria* strains could produce folate which was more than 4000lg/ 100 g dry matter of bacteria. Among selected strains, *Bifidobacterium catenulatum*ATCC 27539 showed the highest folate content, about 9295lg/ 100 g dry matter of bacteria. This study also suggested that folate content of the bacteria depend upon particular bacterial strain, physiological state, and component of medium which leads to maximum folate production per unit biomass (D'Aimmo et al., 2012). Masuda et al. (2012) used nukazuke for extracellular vitamin B12 and thiamine producing lactic acid bacteria isolation. Out of 180 isolated lactic acid bacteria *Lactobacillus sakei* and *Lactobacillus plantarum had* highest folate producing capacity which was near about 100g/L. only two specific strains produced satisfactory level of vitaminB12 that was 2 micro g/ L. study also confirmed that there was no effect of harsh environmental conditions (temperature, salt, pH, ethanol) on folate and vitamin B12 production rate of the specific strain (Masuda et al., 2012). Laiño et al. (2012) isolated Lactobacillus strains, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from artisanal Argentinean yogurts. This present study evaluated extracellular and intracellular folate producing capacity by using nonfat milk and folate free culture medium. The study group observed in folate free culture medium *Lactobacillus bulgaricus*CRL863 *Streptococcus thermophilus*CRL 415 and CRL 803 had maximum extracellular folate producing capacity (22.3 to 135μg/L) in FACM. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* could increase approximately 190% of folate concentration of nonfat milk (Laiño et al., 2012). Crittenden et al. (2003) used yoghurts, fermented milks to isolate common bacteria present into it and choose thirty two strains to detect their folate producing ability. The study used skim milk to detect folate producing capacity during its fermentation. The study showed that some specific strains, including *Enterococcus faecium* and *Streptococcus thermophilus* had folate capacity (Crittenden et al., 2003). Traditional fermented milk namely dadih was used to isolate some potential folate producing strain. The study used Vita fast folate kit for determination of folate production rate. All the 17 isolated *Lactobacillus* strains were able to synthesize folate and the production rate was between 12.43± 3.13 and 27.84 ± 5.80 μg/L (Purwandhani et al., 2018).

CONCLUSION

Several lactic acid bacteria (LAB), such as *Lactobacillus*, are used worldwide for fermentation. Some of the LAB is beneficial for the human health because of the probiotic potentiality. Additionally, few of them can produce vitamins also. This review analyzed the potentiality of *Lactobacillus* to exhibit the vitamin concentration in the tribal fermented foods through incorporation of the microbes into the food samples. This review expected that tribal fermented food derived lactic acid bacteria, which has both probiotic and vitamin producing capacity will be very helpful to encounter several metabolic diseases and micronutrient deficiencies by using as a starter culture for the further fermentation process. Such vitamin producing probiotic *Lactobacillus* would be economically beneficial also.

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