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Emerging Non-Dairy Reservoirs of Probiotic Lactobacillus Species: A Review

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Abstract
Lactic acid bacteria are considered to be probiotic organisms; they provide health benefits when consumed. They are gram-positive, acid-tolerant bacteria. Their utilization in industry is significantly quite high because of the various benefits and low pathogenicity level. They improve the nutritional value of the food, optimizes the lactose digestion, strengthen the immune system. They are used for the preparation of various fermented food worldwide, exemplarily curd, yoghurt, cheese, kimchi, sauerkraut, sausages and pickles. Out of various genera, Lactobacillus is the most well-known among the lactic acid bacteria because of their probiotic properties. It is the largest genus with more than 250 species. Lactobacilli are exploited commercially due to their characteristic probiotic properties. Thus, their isolation and characterization are essential tasks. The sources of isolation of the Lactobacillus can be dairy as well as non-dairy in origin. The dairy sources of isolation are milk and its products. The following article reviews the literature published concerning the isolation of Lactobacillus from non-dairy sources. The sources reviewed were soymilk production waste, fermented food, bees, humans and cows.

Keywords: *Lactobacillus, Probiotic, non-dairy, soymilk, bees and humans etc.*

INTRODUCTION

Lactic acid bacteria play an important role in the food, clinical and agriculture industries. They are a heterogeneous group of bacteria and they are considered safe for consumption, thus, are used extensively for providing nutrition as well as in the health sector. (FAO/WHO, 2002). [1] The core group of the lactic acid bacteria consists of four main genera:-

1. *Lactobacillus*
2. *Leuconostoc*
3. *Streptococcus*
4. *Pediococcus*

In accordance with Khalid (2011) [2], several new genera have been identified as lactic acid bacteria in the recent revision of the taxonomic divisions, those are *Aerococcus, Weissella, Alloiococcus, Carnobacterium, Dolosigranulum, Lactococcus, Enterococcus, Globicatella, Vagococcus, Oenococcus* and *Tetragenococcus.*

As described by Saeed and Salam (2013) [3], lactic acid bacteria are gram-positive, non-spore-forming, non-respiring, cocci-shaped or rod-shaped bacteria (Figure 1). They possess the ability to produce lactic acid as the major end product when fermenting carbohydrates. Other than lactic acid production, these bacteria also degrade proteins and lipids and produce a large variety of compounds such as esters, alcohols, aldehydes and acids. This leads to the development of peculiar flavors in the product fermented by these bacteria.

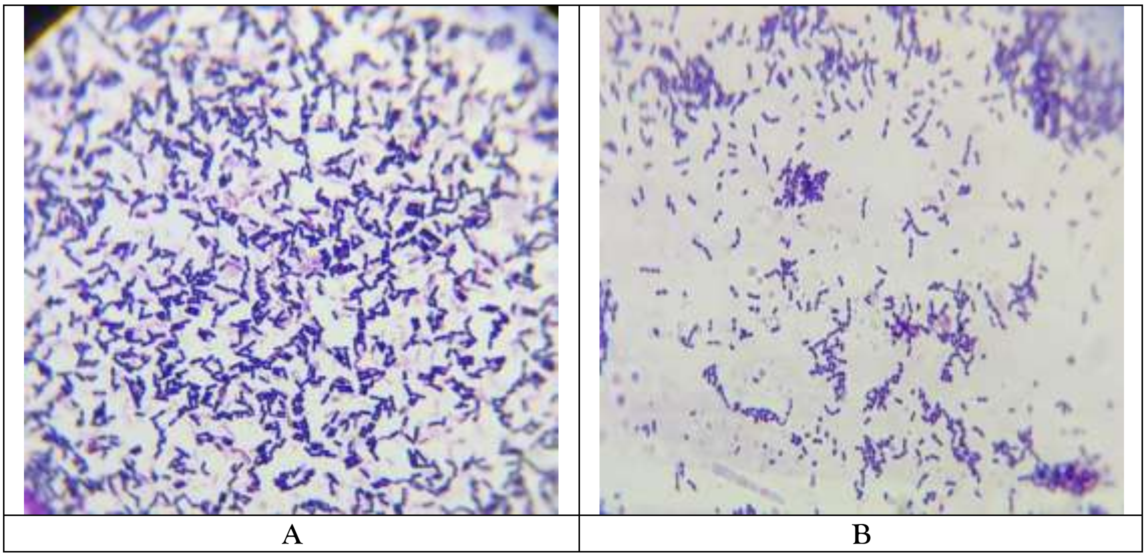

Figure 1: Rod shaped gram positive Bacilli (A) and Cocci (B)

In humans, as well as insects and other animals, lactic acid bacteria are commensal species (Hammes and Hertel, 2006) [4]. They have the ability to modulate immune responses. They can also produce metabolites which are anti-microbial in nature, thus improvising the immune system of the host (Servin, 2004) [5] (Ventura *et al.*, 2009) [6]. Keeping such benefits in mind, Lactic acid bacteria are considered to be probiotics. Probiotics are living organisms which are beneficial to the host organism. For a bacterial strain to be considered a probiotic strain, it must meet a few requirements as mentioned in figure 2. (Zielinska *et al.*, 2015) [7] (Bao *et al.*, 2010) [8] (Zeng *et al.*, 2016) [9] (Ljungh and Wadstrom, 2006) [10] (Ronka *et al.*, 2003) [11]:-

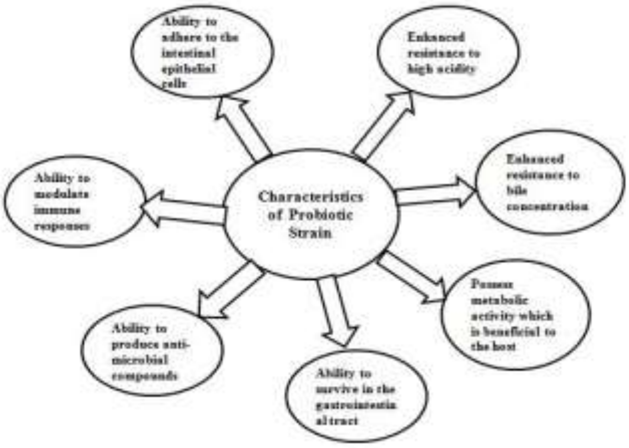

Figure 2: General features of Probiotic Strains.

There are many benefits associated with consuming probiotics. These benefits include (Garcia *et al.*, 2014) [12] (Shukla *et al.*, 2013) [13] (Nualkaekul *et al.*, 2011) [14]:-

- Prevention of atopic allergies
- Healthy gut micro biota
- Improves the digestibility of the food
- Alleviation of lactose intolerance
- Helps in the prevention of diarrhea
- Regulation of blood cholesterol levels
- Controlling hypertension
- Aids in the stimulation of the immune system

Lactic acid bacteria possess these abilities, and thus, are considered to be probiotic. Through various research, it has been found that the potential benefits of consuming lactic acid bacteria on a regular basis include the improvement in the immune function of the consumer, production of bioactive compounds which are beneficial for the health of the consumer as well as protection of the consumer against pathogens. The consumption of lactic acid bacteria also facilitates the digestion and absorption processes in the body.

When fermented by lactic acid bacteria, it is noted that the nutritional quality of the food increases. This is because of the production of bioactive compounds. Numerous studies which have been conducted indicate that the fermentation by the lactic acid bacteria promotes the hydrolysis of the bioactive peptides, and consequently, the production of the bioactive compounds.

Bioactive peptides are those peptide fragments which are inactive until they are hydrolyzed. For example, on hydrolysis, alpha casein and beta-casein liberate more than 20,000 bioactive peptides. (Yamamoto and Takano, 1999) [15]. Rizello *et al.* (2016) [16] observed that the nutritional quality of flour-based food, such as bread, increased when the fermentation was conducted by the lactic acid bacteria.

Lactobacillus is one of the most important genus as well as well-known among lactic acid bacteria. It is the largest genus with more than 250 species and hence is extremely diverse at phenotypic and genotypic levels. This genus was proposed in 1901 by Beijerinck and continues to be expanded with discoveries of new species. It is a genus of non-sporulating, gram-positive, catalase negative, microorganisms. *Lactobacilli are* exploited commercially due to their characteristic probiotic properties.

Lactobacillus such as *Lactobacillus acidophilus, Lactobacillus gasseri, Lactobacillus rhamnosus, Lactobacillus plantarum* and *Lactobacillus fermentum* are probiotic since they possess strain-specific properties which are beneficial to the consumer (Giraffa *et al.*, 2010) [17].

Lactobacillus rhamnosus G.G. is anti-carcinogenic. It decreases the activity of beta-glucuronidase and thus has anti-carcinogenic properties (De Roos and Katan). [18]

Lactobacillus gasseri produces many bacteriocins. One of the most well-known and well-characterized among these bacteriocins is Gassericin A, produced by *Lactobacillus gasseri*LA39 which was isolated from feces of infants. (Toba T, 1991). [19]

Another *Lactobacillus* species- *Lactobacillus acidophilus* L1 was reportedly found to be able to reduce the serum cholesterol levels. This indicated the potential of this bacterial strain to reduce the risk for coronary diseases by 6% to 10% in people who are hypercholesterolemic (Anderson and Gilliland., 1999). [20]

Keeping all these benefits of *Lactobacillus* in mind, it is generally suggested to include its potential sources in the daily diet. Milk and its products are considered to be the best source of *Lactobacillus*.

However, despite all these benefits, the consumption of milk based diet is limited due to the allergies associated to it, cholesterol imbalance, dyslipidemia and vegetarianism (Perricone M, 2015) [21]. Hence, to cater the needs of the larger scale of population, isolation and characterization of *Lactobacillus* from non-dairy sources is in demand.

Researchers have achieved success in this endeavor as well. *Lactobacillus* has been isolated from various non-dairy sources such as fermented food, animals, humans, insects, fruits etc. Surplus production has been reportedly found from various substrates such as molasses, whey, cassava bagasse, hydrolysate newspaper, sweet sorghum juice, lignocellulosic biomass (Zhang and Vadlani, 2015) [22] (Wee *et al.*, 2006) [23] (Abedi and Hashemi, 2020) [24].

Lactobacillus from soy milk

Soy milk is a functional food which is widely consumed by people all around the world. It is widely popular among lactose intolerant patients as well as those individuals who are in the search of a good substitute of milk due to vegetarianism. Soy milk contains probiotic bacteria like lactic acid bacteria. After its production, a large amount of waste product is also produced called okara (Suruga K, 2011) [25]. It is the residues which have been left behind after the pureed soybeans have been filtered. The waste product is not fit for consumption and thus, is of low economic value. It is also a source of pollution because it is directly dumped in open since it cannot be used.

However, despite its low economic value, this waste product can be used for isolation of *Lactobacillus* as it is a good source.

One of the main reason behind its potential candidacy as the medium for LAB isolation is that it contains nutritional values which is an important factor for LAB production. Okara contains nutrients such as proteins, dietary fibers, fats, oligosaccharides, monosaccharides. These are important for the callus growth of LAB. Aritonang *et al.* (2017) [26] isolated and identified LAB with probiotic properties using okara as a medium. Along with that, they analyzed its antimicrobial activity against pathogens like *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* EP01,The methods used to examine the isolates were same as the methods used by Felten *et al.* (1999) [27] . Rossi *et al.* (2021) [28] isolated *Lactobacillus* from the waste product of soy milk production. Again *Lactobacillus plantarum* was the species which was found predominantly in the okara samples. Steven Manzi *et al.* (2015) [29] isolated 28 bacterial strains from spontaneously fermented soy milk. The strains were assessed for their probiotic characteristics like low pH resistance, bile salt resistance, antibacterial activity and hemolysis test.

Lactobacillus from Domestic Cattles

Domestic Cattles like Cows are also a source of *Lactobacillus* and various species have been isolated from the cows. The microbial community residing in the gut of ruminants plays a key role in the health, nutrient digestibility in the cattle (Guarner and Malagelada, 2003).[30] By ensuring healthy gut microbiota, the milk yield can also be increased. Nitrogen-based pollutants and methane production can also be reduced with the help of healthy, probiotic microorganisms (Strohlein, 2003). [31] Several studies have been done which provides evidence to support this statement. One such study was published in January 2020 (Guo *et al.*, 2020) [32], in which Lactic Acid Bacteria were isolated from the rumen fluids and feces of dairy cows. Scientists were able to isolate *Lactobacillus sp.* from the samples. Six Holstein dairy cows were selected, aged 100-200 days. Their care was done accordingly to the guidelines outlined by the Guide for Care and Use of Agriculture Animals in Research and Teaching (2010, FASS) [33]. They were fed same amount of alfalfa silage and their rumen fluids and feces samples were collected afterwards, total 104 strains were isolated from these samples. Those strains which were fast growing and were capable of high acid production were further assayed and 16S rRNA gene sequencing was done to identify them at species level. Four such strains were identified out of the 104.

These four strains were:-

1. F1
2. F50
3. L100
4. L120

F1 and F50 were isolated from the feces samples. These two were identified to be *Lactobacillus plantarum.* Whereas L100 and L120 were isolated from the rumen fluids. L100 was identified to be *Lactobacillus salivarius* and L120 was *Lactobacillus fermentum.* They showed more than 99% 16S rRNA sequence similarity. The strains L100, F1 and F50 were gram positive, catalase negative and rod-shaped homofermentative bacteria whereas the strain L120 was gram positive, catalase negative and rod-shaped heterofermentative bacteria. It showed growth between the temperature ranges of 15 °C to 40°Celsius. The rest three showed growth at 5°C to 45°C. Adetoye *et al*, (2019)[34] and Lin *et al.* (2020)[35] isolated *Lactobacillus* species (*Lactobacillus reuteri, Lactobacillus salivarius, Lactobacillus gasseri*) from the fecal samples of dairy cows and cattle feces.

Lactobacillus from Insects

Insects including honey bee, wild bees and bumble bees were reported to source of lactobacillus

There exists a close relationship between the insects and the microorganisms which inhabit their specialized cells, organs or guts (Boutzis and Miller, 2003).[36] The microflora which inhabits the intestine facilitates assimilation of nutrients and immune functions. (Sandine, 1979) [37]

As suggested by Dillon and Dillon (2004) [38], in the insect gut, the growth of the pathogenic microbes is inhibited by the insect gut microbiota and insect gut is an organ which sports high microbiological diversity.

The bees belonging to the families Megachilidae and Halicitidae were included in the experiment. Following species of bees were included:-

- *Agapostemon* species
- *Megachile policaris*
- *Halictus ligatus*
- *Halictus tripartitus*
- *Augochlora pomoniella*
- *Dialiticus* species

The flowers used for the experiment was of *Abutilon* species. The wild bees, unlike the honey bees or bumble bees, interacts and harbors a wide range of *Lactobacillus* which go undescribed (McFrederick QS and Rehan SM, 2016) [39] (McFrederick QS, 2014) [40] (McFrederick *et al.*, 2017) [41] (McFrederick *et al.*, 2012) [42].

From those pure isolates, DNA extraction was done. The extraction of the genomic DNA, PCR of the DNA targeting the 16S rRNA gene and the sequencing of the PCR products- all of this was done according to McFrederick *et al.*, 2017. [41] and three novel strains belonging to the *Lactobacillus* genus were isolated and identified in this experiment. They were named after the famous bee biologists. The strains obtained were:-

- *Lactobacillus quenuiae*
- *Lactobacillus timberlakei*
- *Lactobacillus micheneri*

The gut of the honey bee harbors various lactobacilli. These lactobacilli are divided in two clades, called Firm 4 and Firm 5. (Moran NA, 2012) [43] (Engel P, 2012) [44] (Anderson KE, 2016) [45].

In the Firm 4 clade, two *Lactobacillus* species are included. These species are *Lactobacillus mellifera* and *Lactobacillus mellis.* In the Firm 5 clade, four *Lactobacillus* species are included. These species are *Lactobacillus kimbladii, Lactobacillus kullabergensis, Lactobacillus melliventris* and *Lactobacillus helsingborgensis.*

Together, these two clades form another clade which is called *Lactobacillus delbruekckii*clade. (Olofsson TC, 2014) [46]. Other than these two clades, other *Lactobacillus* species are also found in the bees. The most predominant species of such *Lactobacillus* species is *Lactobacillus kunkeii.* It is found mostly in the *Apis* species of the bees. It is reported by several researchers. (Vásquez *et al.*, 2012) [47] (Tajabadi *et al.*, 2011, 2013b) [48] [49] (Corby *et al.*, 2014) [50] (Anderson *et al.*, 2013) [51] (Endo *et al.*, 2012) [52]*Lactobacillus kunkeii*was isolated from *Apislaboriosa,* a species of bee found in Nepal, and the colonendeavourg units per gram of honey was 10^8. This was followed by isolation of the species from *Apis mellifera* honey. The species *Apis mellifera* is found in Africa. The stomach of *Apisdorsata* also harbors this species. (Tajabadi*et al.*, 2011) [48] .This species is associated with the honey bee foregut.

Lactobacillus has also been isolated from Asian dwarf honey bee, *Apis florea.* This species of bee is distributed over a wide range of 7000 km.

A strain closely related to *Lactobacillus pentosus*was also isolated from the stingless bees found in Sabah, North Borneo. These bees are found in the tropical rainforests. (Eltz T., 2003) [53]. Thirty five stingless bee species are found in Peninsular Malaysia and thirty two in Borneo (Schwarz HF, 1937) [54] (Jaapar M., 2016) [55].

Other associated lactic acid bacteria found in the bees are:-

1. *Lactobacillus johnsonni* (Carina Audisio M, 2011) [56]
2. *Lactobacillus plantarum*(Mudronová D, 2011) [57]
3. *Lactobacillus brevis* (Mudronová D, 2011) [57]
4. *Lactobacillus Apis* (Killer J, 2014) [58]

The above mentioned *Lactobacillus* species exhibit inhibitory action against *Paenibacillus larvae* and *Mellisococcus plutonis.* The *Lactobacillus delbruekckii* clade found in honey bee gut is vital for their health as it helps the worker bee to gain weight. It does so by producing short chain fatty acids. (Zheng H, 2017) [59]

Thus keeping that in mind, many researches have been done to isolate *Lactobacillus* from the bees. Praet *et al.* (2015) [60] has isolated *Lactobacillus* from the bee gut which showed 97.0% similarity with *Lactobacillus apis.* Later, further processing identified this *Lactobacillus* species to be a novel species and *Lactobacillus bombicola*was proposed as its scientific name.

A research was conducted by Parichehreh *et al.*[61] and their research was published on 5th of April 2018. Isolated *Lactobacillus* from the wild species of *Apisflorea*. This wild species is found in a wide range- Vietnam and southeast China, South of Himalayas, plateau of Iran, South of Oman (Hepburn *et al.*, 2005) [62]. Ruttner *et al.* (1988) [63] reported three morpho clusters of this species. These are- South India and Sri Lanka (Bourtzis and Miller, 2003) [36], Thailand, Oman and Pakistan (Chung *et al.*, 1989) [64].

Thus, the range of distribution of this species is around 7000 km. It was noted that the dominant lactic acid bacteria in honey stomach of the bees changed when the sampling was done from different flowers during the year (Olofsson and Vásquez, 2008) [65]. This study was done with the aim to detect and identify lactobacilli residing in the gastrointestinal tract of *Apis florea* collected from the different regions of Iran. Another research was done by Goh *et al.*, (2021) [66]. Isolation and characterization of lactic acid bacteria isolated from the stingless bees found in Sabah, North Borneo was done for food and probiotic application. In this experiment, total seven potential antimicrobial lactic acid bacteria isolates were isolated and they had an inhibitory action against *Listeria monocytogenes* ATCC 7644. To check the antimicrobial activity, indicator strains were used. Those indicators were:-

1. *E. coli* ATCC 11775
2. *L. monocytogenes* ATCC 7644
3. *Salmonella enterica* ATCC 13076

They were also able to survive the conditions of gastrointestinal tract such as- Acidity, Bile, Temperature and Salt.

Lactobacillus from agricultural products:-

Lactobacillus is also found in abundance in the variety of agricultural products like raw carrots, french beans, marrows (Cagno *et al.*, 2008) [67], yellow passion fruit (Sari *et al.*, 2013) [68] purple passion fruit (Fatimatuz Zahro, 2014) [69], soybean waste (Malik *et al.*, 2008) [70] , red and yellow peppers (Cagno *et al.*, 2009) [71], African cereals Acha and iburu, durian (Ahmad *et al.*, 2018) [72], cocoa (Lefeber *et al.*, 2011) [73] (Lefeber *et al.*, 2012) [74], mango (Arsyik*et al.*, 2015) [75], cereal foods (Oguntoyinbo and Narbad, 2015) [76]. Fruits and their products have been found to horde several species of *Lactobacillus*. The consumption of the fruits and their products has increased due to the growing recognition among the public about their nutritional value such as high content of minerals, vitamins and secondary phytochemical compounds (Rufino *et al.*, 2010) [77] (Silva *et al.*, 2014) [78]. Strains closely related to *Lactobacillus plantarum* subsp. *plantarum* and *Lactobacillus pentosus* has been identified and isolated from the fermented cocoa beans. In some of these strains- when their supernatants were prepared- they exhibited the ability to inhibit the growth of the pathogenic strains of *E.coli*, *Bacillus subtilis* and *Staphylococcus aureus*- even after they were neautralized (Fahrurrozi*et al.*, 2019) [79]. Wu *et al.*, (2018) [80], isolated lactic acid bacteria from pineapple waste. Upon isolation *Lactobacillus* was found to be the most dominant species. 12 lactic acid bacteria morphotypes were isolated from the pineapple waste. 16S rRNA and pheS genes used to identify the Lactic acid bacteria by their amplification and sequencing. Two different genres of lactic acid bacteria were identified. These were *Lactobacillus* and *Weisella* species. *Lactobacillus* was found in the most abundance as 11 strains of *Lactobacillus* were found.

This was in accordance with the trend of the other reports of bacterial isolation from fruits and vegetables, since the previous reports also concluded that the *Lactobacillus* is the most abundant and is characterized by its exceptional size and genetic diversity (Torino *et al.*, 2015) [81].

The two different species of *Lactobacillus* which were found were:-

- *Lactobacillus parafarraginis*- two isolates
- *Lactobacillus fermentum*- two isolates

Besides these four isolates, the other seven isolates were grouped with the *Lactobacillus casei* and *Lactobacillus paracasei.* To identify and group these stains with these two species— Multilocus Sequence Typing (MLST) genes were used. Pineapple waste was used by Pyar*et al.* (2014) [82] to isolate *Lactobacillus* since the medium held the potential to encourage the growth of *Lactobacillus.* It was observed that the growth on the pineapple waste was comparable to the growth in MRS agar media. Much like pineapple, strawberries can also be used for *Lactobacillus* isolation. Fevria*et al.* (2019) [83] was able to isolate *Lactobacillus* from strawberry. The samples for the experiment were collected from the community plantations in Angek, Tanah Datar district. Ngouénam*et al.* (2021) [84] isolated *Lactobacillus* from overripe fruits and fruit derived by products. From the market of Dschang, Menoua Division, West Cameroon, 56 samples of overripe fruits were collected aseptically from March to June, 2018. These fruits were oranges, banana, papaya and pineapple.

These 5 different isolates of *Lactobacillus* were:-

- *Lactobacillus brevis*
- *Lactobacillus pentosus*
- *Lactobacillus paracasei*

- *Lactobacillus plantarum*
- *Lactobacillus fermentum*

Much like raw fruits, pickles prepared from fruits as well as vegetables are also a rich source of *Lactobacillus*. Pickling is one of the oldest methods of food preservation and has been practiced widely all around the world. Besides the preservation of food, pickling also increases the nutritive value of the food. Pickles also acts as condiments as well as palatability enhancers (Joshi and Bhat, 2000) [85].Arindam Roy and Chandan Rai (2017) [86] isolated lactic acid bacteria from pickles. 14 different types of pickles were chosen and 50 samples of these pickles were collected from the retail shops, vendors as well as households from Kolkata, India. These samples were collected in sterile sampling bags and they were kept in ice box for transporting them in the laboratory for isolation.These 5 LAB isolates were gram positive, catalase negative and rod-shaped. By API 50 CHL kit, these isolates were identified as *Lactobacillus* species.

- LABM1- *Lactobacillus fermentum*
- LABM2 and LABM3- *Lactobacillus pentosus*
- LABM4 and LABM5 *Lactobacillus paracasei*

Another research was done for the isolation of lactic acid bacteria found in the traditional pickles of Himachal Pradesh, India by Monika *et al.* (2017). [87] In Himachal Pradesh, besides from the generally used ingredients for pickling, black mustard is also used- which gives the pickles made in Himachal Pradesh a traditional characteristic flavor. The collection of sample was done from the rural as well as the urban areas of Himachal Pradesh. In 2020, Prakash *et al.*[88] isolated *Lactobacillus fermentum* strains from lemon pickle as well as rice water. The lemon pickle was obtained locally from Amritpuri, Kollam, Kerala, India. The rice water samples were obtained from cooked rice and the cooked rice was fermented aseptically for 6 hours. The samples were serially diluted up to 10^{-5}. 96 well plates was used and 100 ul of 10m/ml gelatin was placed in each well. The well played was incubated for one hour and then it was raised with phosphate buffer solution thrice. The isolates were suspended in the phosphate buffer solution and they were kept in the well plates at room temperature. After 10 minutes, the supernatants were discarded and 100 µl phosphate buffer solutions were used to wash it 5 times. The 5th wash was plated. 1N NaCl was added to PBS and the plates were again washed. Then, 1% dimethyl sulfoxide (DMSO) was added to phosphate buffer solution and the well plates were again washed five times. Then, it was plated in the MRS agar plate. From the 1% DMSO and phosphate buffer saline solution's 5th wash, uniform pinheaded colonies were obtained. These colonies were sub cultured for characterization. For the genomic DNA isolation, the phenol chloroform method was used (Green and Sambrook, 2017) [89] (Porayath *et al.*, 2017) [90]. 16S rRNA gene was targeted during the PCR sequencing and the PCR products were then analyzed using BLAST software. The isolates obtained from both the rice water and the lemon pickle was *Lactobacillus fermentum*. The strains isolated from these two were deposited in the GenBank, under the following assession numbers:-

- MN410703- Rice water isolate
- MN410702- Pickle isolate

Fermented rice is also a good source of *Lactobacillus.* However, it is one of the neglected foods, even in the countries where rice is a staple food, despite the associated health benefits. Jeygowri *et al.*, (2015) [91] studied the isolation of potentially probiotic *Lactobacillus* species from fermented rice. For samples, red rice and white rice samples were purchased from the local market. 3 types of agar were used for the isolation of *Lactobacillus*. The 3 types of agar used were as follows:-

- MRS agar
- MRS agar supplemented with 0.1 to 0.2% sorbitol
- MRS agar supplemented with 0.25% L-cysteine.

MRS agar supplemented with sorbitol was used since it interferes with the growth of *Staphylococcus*, *Bacillus* and yeast species (Janet *et al.* 2012) [92]. It is also selective for *Lactobacillus* species (Tharmaraj and Shah, 2003)[[93]. The MRS agar supplemented with L-cysteine is selective for *Lactobacilli* and *Bifidobacteria* (Haddadin *et al.*, 2004) [94]. The pH of the agar medium used was as follows:-

- MRS agar- 6.2
- MRS L-cysteine- 5.8
- MRS sorbitol- 6

For the preparation of the sample, 75 grams of red raw rice and 75 grams of white raw rice were taken and cooked with water in the rice : water equivalent to 1:3 respectively. It was cooked for 30 minutes. 50 grams of cooked and raw rice was taken in a 250 ml clay pot and soaked overnight, which is 12 to 16 hours, in sterile distilled water at the temperature 27 °C. The ratio of rice to water was maintained as 1:3 respectively, 10 ml of the fermented rice steep of both white and red rice, cooked and uncooked, was collected separately and it was vortexed. Then the vortexed solution was diluted in 0.85% sterile saline solution up to 10^{-3} dilution. 100 ul of the diluted as well as the undiluted sample was taken and inoculated on the petri plate with the help of spread plate method. These plates were incubated for 3 days anaerobically at 37 °C. After the incubation period was over, the colonies of gram positive and catalase negative bacilli were subcultured by streaking them and this was done 4 to 5 times to ensure the purity of the culture. The pure isolates obtained were preserved in MRS broth and 20% glycerol at -80 °C. For the identification of the isolates, 5 methods were employed. These methods were as follows:-

- Colony Morphology observation
- Gram staining
- Catalase test
- Motility test
- Endospores test

Isolated species of the *Lactobacillus* was as follow:-

- *Lactobacillus helveticus*
- *Lactobacillus curvatus*
- *Lactobacillus delbrueckii ssp.*
- *Lactobacillus plantarum*
- *Lactobacillus pentosus*

Lactobacillus has also been isolated from corn silage by anaerobic culturing (Kasra *et al*, 2010) [95].The experiment was done in Iran and the samples were taken from a cold region of Iran. For the purpose of increasing the stability of the microorganism in the harsh environment, the method of microencapsulation was employed. The stabilizing polymers used for microencapsulation were alginate and chitosan. After the isolation of the microorganisms from the corn silage, they were identified with the aid of bacteriological and biochemical methods. The microorganisms isolated were identified to be of *Lactobacillus* genre.

Isolation of *Lactobacillus* from kefir samples has also been done. Kefir is a home-made, naturally fermented food product. It is a beverage produced by fermenting the kefir grains. It is consumed frequently in Japan, Malaysia, United States of America, China, France and Brazil. Kefir has been a part of diet for a long time and some its properties include (Sharifi *et al.*, 2017) [96] :-

- Acidic taste
- Sour taste
- Easily digestible
- Slightly alcoholic

The fermented beverage has been known for possessing probiotic characteristics such as anti-obesity, anti-inflammatory, anti-oxidative, anti-microbial, anti-tumor (Kim. *et al.*, 2017) [76] (Yilmaz *et al.*, 2018) [98] (Bengoa *et al.*, 2018) [99] . Its microflora is complex and contains a variety of probiotic bacteria and yeast. Out of all the Lactic Acid Bacteria, *Lactobacillus* is the most dominant group found in kefir (Gao, 2017) [100]. Talib *et al.* (2019) [101] isolated *Lactobacillus* from Malaysian kefir grains.

These identified *Lactobacillus* strains are as follows:

- *Lactobacillus harbinensis*
- *Lactobacillus plantarum*
- *Lactobacillus paracasei*

Kimchi is another fermented food which is rich in *Lactobacillus.* It is traditionally made and consumed in Korea and constitutes of fermented vegetables and various seasonings. Due to the presence of lactic acid bacteria, it is nutritious and possesses probiotic properties. The most prominent out of the lactic acid bacteria present in the kimchi is *Lactobacillus sakei* (Jeong *et al.*, 2013)[102]. This species of *Lactobacillus* was found to be able to modulate TH2 immune responses. It also has immune regulatory effects since it can increase the production of anti-inflammatory cytokine (Hong Y.F., 2014) [103]. Won *et al.* (2020) [104] also isolated lactic acid bacteria from kimchi. 225 isolates in total were isolated from the kimchi samples. Out of these 225 samples, 15 were selected for screening. For these 15 isolates, to identify the isolates, 16S rRNA gene sequencing was done.

The *Lactobacillus* species identified were:-

- *Lactobacillus plantarum*
- *Lactobacillus sakei*

Yoseph Asmelash Gebru and Desta Berhe Sbhatu (2020) [105] isolated *Lactobacillus* from the samples of kimchi, teff batter and teff grains. Teff grains are staple food in Ethiopia and their fermentation is mainly done with the aid of lactic acid bacteria (Fischer *et al.*, 2014) [106]. They are beneficial for consumption since they are gluten free as well as rich in essential amino acids and minerals (Zhang *et al.*, 2016) [107]. Thus, isolation of *Lactobacillus* from such source of food increases the importance of the staple food and gives us an insight in the probiotic properties of the food.

These isolated species was:-

- *Lactobacillus brevis*- 10 isolates
- *Lactobacillus plantarum*- 9 isolates

Another fermented product which has been widely known to harbor *Lactobacillus* is suaerkraut. It is consumed in Eastern and Central Europe and has been part of the diet since Roman Empire because it served as the source of nutrition during the harsh season of winter when the shortage of food persisted. Sauerkraut is a fermented cabbage food product which contains 2.5% salt. Other than *Lactobacillus*, various other lactic acid bacteria are also involved in its fermentation. These lactic acid bacteria include *Leuconostoc* and *Pediococcus*. The fermentation of sauerkraut occurs in two phases (Zabat *et al.*, 2018) [108]

- Heterofermentative phase- *Leuconostoc mesentroides*
- Homofermentative phase- *Lactobacillus plantarum*

Touret *et al.* (2018) [109] also isolated *Lactobacillus* from the sauerkraut. For this experiment, 4 different sauerkraut samples were prepared. Two different types of cabbages were used:-

- Portuguese cabbage or *Brassica oleraceavariety costata*
- Pointed head cabbage or *Brassicaoleracea* variety *capitata*

For each type of cabbage, two different recipes were followed for sauerkraut fermentation:-

- Only salt used.
- Salt, with the addition of aromatic herbs such as laurel, rosemary, thyme, garlic and lavender.

Lactobacillus from humans:

Humans are also a source of *Lactobacillus,* in infants, beneficial and probiotic bacteria are found in abundance (Rodriguez *et al.*, 2015) [110] (Kook S-Y, 2018) [111] . Jomehzadeh *et al.*, (2020) [112] isolated *Lactobacillus* species from infants. For sample collection, 120 infants aged less than 24 months were selected. There fecal samples were collected and were inoculated in MRS broth. The samples were cultured anaerobically at 37 °C for 48 to 72 hours. After the incubation period was over, colonies were picked out on the basis of their morphological characteristics. For these colonies, pure cultures were obtained by repeated streaking on MRS agar plates. Phenotypic tests were conducted for the identification of *Lactobacillus.* The phenotypic tests conducted were as follows (Kilic and Karahan, 2010) [113] (Davoodabadi *et al.*, 2015a) [114]:-

The strains identified were as follows:-

- *Lactobacillus plantarum*- 9 strains-30% (N12 to N20)
- *Lactobacillus fermentum*-11 strains- 36.7% (N1 to N11)
- *Lactobacillus rhamnosus*- 6 strains- 20% (N21 to N26)
- *Lactobacillus paracasei*- 4 strains- 13.3% (N27 to N30)

Lactobacillus fermentum was the most prevalent strain.

Oh *et al.* (2018) [115] isolated and identified *Lactobacillus* species from the fecal samples of infants. For the fecal sample collection, 5 healthy infants, aged 2 weeks or younger, were selected. The fecal samples were obtained directly from the diapers of the healthy infants.

With the help of the MALDI TOF mass spectrometry, the strains were identified at species level. 3 *Lactobacillus* species were identified. These species were as follows:-

- *Lactobacillus reuteri*

- *Lactobacillus gasseri*
- *Lactobacillus casei*

The various strains and their identification was as follows:-

- 3MO2- *Lactobacillus reuteri*
- 3MO3- *Lactobacillus reuteri*
- 4M13- *Lactobacillus gasseri*
- 4R22- *Lactobacillus gasseri*
- 5R01- *Lactobacillus gasseri*
- 5RO2- *Lactobacillus gasseri*
- 5R13- *Lactobacillus gasseri*
- 4B15 *Lactobacillus casei*

Lactobacillus gasseri was the most prevalent strain among the other *Lactobacillus* species.

Similarly, Kook *et al.* (2019) [116] isolated probiotic strains of *Lactobacillus* from infant and children feces. These genera are:-

- *Bifidobacterium*
- *Lactobacillus*
- *Klebsiella*
- *Staphylococcus*
- *Enterococcus*
- *Streptococcus*

Lactobacillus has also been isolated from the saliva of healthy individuals (Hirasawa M and Kurita-Ochia T., 2020) [117] isolated potentially probiotic *lactobacilli* from the saliva of periodontally healthy individuals. *Lactobacillus* was identified on the basis of the colony and cell morphology. The selected colonies were then transferred to Rogosa SL agar and it was incubated under anaerobic conditions for 24 to 48 hours. For the purification of the isolates, repeated streaking was done. Further gram staining and catalase activity was checked for the identification of the *Lactobacillus*. Those isolates which were gram positive and catalase negative were then identified using 16S rDNA sequencing. Total 200 putative *Lactobacillus* strains were obtained. K1-2, K1-5, K1-6-2, K1-7, K1-8 and O3-2 were gram positive, rod-shaped strains which did not possess capsules. The 16S rDNa sequencing identified these strains as follows:-

- K1-2 K1-7 and K1-8— *Lactobacillus casei*
- O3-2— *Lactobacillus gasseri*
- K1-5 and K1-6-2 — *Lactobacillus fermentum*

Lactobacillus has also been found to be harbored in the human vagina. Pino *et al.* (2019) [118] isolated various species of *Lactobacillus* from the human vagina. The samples were taken from 30 volunteering participants. All the participants aged between 18 to 36 years old. All the participants had regular menstrual cycle and healthy vagina cytology. The samples of the vagina discharge were collected and analyzed.

Total 300 isolates were found and out of these 300 isolates, 261 isolates belonged to *Lactobacillus* genius. 8 species of *Lactobacillus* genus were found. The species are as follows:-

- *Lactobacillus gasseri-* 28%
- *Lactobacillus plantarum-* 1%
- *Lactobacilluss alivarius-* 20%
- *Lactobacillus crispatus-*18%
- *Lactobacillus helveticus-*13%
- *Lactobacillus paracasei-* 1%
- *Lactobacillus fermentum-* 10%
- *Lactobacillus rhamnosus-*10%

Parolina *et al.* (2015) [119] isolated *Lactobacillus* from the mid vagina secretions or discharge. For this experiment, 15 premenopausal volunteers were selected. All the volunteers were of Caucasian race and aged 18 to 45 years old. The volunteers showed no symptoms of urinary tract infection or vaginal tract infection. When these samples were taken, all the volunteers were non-menstruating as well as they were not receiving any kind of antimicrobial treatment, whether oral or local, for the past 2 weeks.

The 16S rRNA gene sequencing identified the isolates to be of the following species:-

- *Lactobacillus crispatus-* 8 isolates – BC 1 to BC 8
- *Lactobacillus gasseri-* 6 isolates- BC 9 to BC 14
- *Lactobacillus vaginalis-* 3 isolates- BC 15 to BC 17

CONCLUSION

Safe to say, *Lactobacillus* is considered to be one of the most important probiotic microorganisms. It has been given the status of GRAS (Generally recognized as safe) since it is non-pathogenic and hence, poses no threat to humans, animals, plants or the environment, in general. Its ubiquitous availability and non-pathogenicity spiked the curiosity of early researchers. *Lactobacillus acidophilus* was first isolated by Dr. Ernst Moro, in 1890, from infant feces. Following this discovery, many scientists studied the Lactobacillus genera extensively. In broad terms, there are two sources of *Lactobacillus* isolation— dairy and non-dairy. Isolation from dairy is traditional. However, in recent years, the non-dairy sources have also been gaining recognition for isolation. The recent exploration is due to the diverse metabolic profile of the strains isolated from the non-dairy sources as well as the unique flavor they can provide. Besides this, due to the ascending trend of veganism as well as keeping the lactose intolerant individuals in mind, it becomes important for the industries to come up with an alternative. This quest has been rewarding one as well. Many sources of *Lactobacillus* have been discovered— humans, naturally fermented food products, bees, silage, plants etc. Some strains, such as those isolated by Akinniyi Osuntoki and Ifeoma Korie (2010) [120] from Nigerian fermented food had high radical scavenging capacity, which could be used for anti-oxidative purposes. Sadeghi (2016) [121] isolated *Lactobacillus plantarum* from pickled garlic. The strain isolated possessed the survival rate of 36.30% in pH 2 and 66.08% in 0.3% bile salt. It exhibited antimicrobial activity against *Listeria monocytogenes* and was resistant to various antibiotics. Those antibiotics were:-

- Norfloxacin
- Vancomycin
- Nalidixic acid
- Streptomycin
- Kanamycin

Darsanaki *et al.* (2012) [122] isolated *Lactobacillus* from vegetables. Three species of *Lactobacillus* were isolated from the samples:-

- *Lactobacillus plantarum-* 3 isolates
- *Lactobacillus casei-* 2 isolates
- *Lactobacillus brevis-* 1 isolate

These isolates had high acid tolerance. They were also able to survive in the presence of high concentration of bile salts and exhibited antimicrobial activity against *Staphylococcus aureus* PTCC 1431, *Salmonella typhimurium* PTCC1639 and *Escherichia coli* PTCC1399. *Lactobacillus reuteri* isolated from the mammalian ileum, jejunum and rectum produces compounds like reuterin, lactic acid etc. which are anti-microbial in nature and helps in the remodeling of the microbiota of the host. Also, the *Lactobacillus gasseri* isolated from the vaginal tract of the cattle is capable of inhibiting the *Staphylococcus aureus* by the production of lactic acid and hydrogen peroxide. Thus, the *Lactobacillus* isolated from various non-dairy sources differ from the dairy sources in terms of antibiotic properties, bile tolerance, acid tolerance, temperature tolerance etc. This difference is due to the difference in the raw material used in isolation.

Some studies show that the isolated from non-intestinal sources tend not to produce bacteriocin or bacteriocin-like substances. Their anti-microbial activity is the virtue of the presence of compounds other than bacteriocin, like lactic acid, hydrogen peroxide etc. Undoubtedly, *Lactobacillus* isolated from the non-dairy sources present to us a new play field to search for the diversity of strains which possess unique characteristics. These characteristics can be used and exploited commercially for the betterment of various industries such as cattle, pharmaceutical, agriculture.

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