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REVIEW ARTICLE

Probiotics: A Promising Tool for Calcium Absorption

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Abstract: As we know nutrients are necessary for the development and proper functioning of the human organism. Bioavailabilities of nutrients are the major concern rather than the supply of an adequate amount of nutrients in the diet. Many of the researches have been shown that the consumption of probiotics along with dairy foods buffers the acidity of the stomach and increases the bacterial survival rate into the intestine. A dairy product with probiotics also provides many of essential nutrients, including protein and calcium. From all the necessary nutrients the calcium having a major role in the human body including the development of bone and teeth are also regulating enzymes and many more. Calcium is the most essential nutrient, about 99% of calcium found in teeth and bone in the body and only 1% is found in serum. A numbers of researches have shown that adequate amount of calcium intake leads to reduce risk of fracture, Osteoporosis and Hypoglycaemia and diabetes in some population. Many of the researches suggested that the Probiotics having a significant role in improvement of calcium uptake and absorption, hence the present review gives information about the relationship of probiotics and calcium, ensuring higher bioavailability of calcium and promising a better bone health. Here, the review study showed a significant role of probiotics in calcium absorption and thus the bioavailabilities. Moreover, it is focused on glimpse of various studies and in-vitro models associated with the phenomena of calcium absorption and uptake.

Keywords: Probiotics, Importance of calcium, LAB, Calcium bioavailability, Bone health, Hypoglycaemia.

1. INTRODUCTION

1.1. Historical View

The origins of yogurt are unknown, but the ancient records had mentioned the use of fermented milk for health benefits known from Vedic time as per record in Indian Ayurveda from about 6000 BC [1]. Also, few records mentioned that the yogurt was invented in Mesopotamia in 5000 BC. The term probiotics was first introduced by Nobel laureate Metchnikoff (1907), he hypothesized that regular consumption of yogurt responsible is for long life. After the publication of the book entitled: "The prolongation of life" by Elli Metchnikoff-1908 boosted the scientific interest in the area of probiotics. The first clinical trial was carried out in the 1950s to study the effect of probiotic on constipation and a probiotic product was licensed by US department of Agriculture as a drug to treat E.coli infection which termed as scour among pigs [2]. As per WHO Probiotics is 'live microorganism which when administered in adequate amount confers a health benefit on the host'. The human body consists of 10 million cells, but we harbor 100 million bacteria in different parts of body [3].

From the past century the probiotics proved their scientific efficacy towards human and animal health by reducing symptoms of lactose intolerance, boost the immune system, enhancing the synthesis of vitamins and bioavailability of nutrients Table **1**, improving the gut health, and decreases the susceptibility towards the allergy, reducing the risk of cancer [4].

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2. IMPORTANCE OF CALCIUM IN HUMAN HEALTH

As per one of the surveys (during the period of 1963-2005) this report was published in Indian Journal of medical research 2008 under the title Nutritional Bone disease in Indian Population stated about 337.68 million population from 0.39 million villages in 22 states of India. The Report showed that more than 4 lakhs patients indentified with the bone and mineral metabolism disorders, more than 20 thousand patients with the nutritional bone disease and metabolic bone disease, more than 17 thousands had been identified with endemic skeletal fluorosis and in 41 patients with the unidentified rare bone disease. This extensive long termed survey had shown that how adequate calcium intake as well as calcium absorption is necessary. Calcium has a vital role in the development of bone and teeth about 90% of calcium involves in the development of bone including formation and physiological strength of skeletal tissue Muscle contraction, Blood coagulation (factor IV), Nerve transmission, Membrane integrity and permeability, Activation of enzymes and in the release of hormones *etc* [5, 6].

Table 1. Commonly used probiotics species for human use [4, 75, 76].

Lactobacillus	Bifidobacterium	Streptococcus	Enterococcus
L.acidophilus	*B.bifidum*	*Strep.cremoris*	*E.faecium*
L.rhamnosus	*B.breve*	*Strep.diacetylactis*	*E.faecalis*
L.brevis	*B.bifidus,*	*Strep.intermedus*	–
L.gallinarum	*B.adolescentis*	*Strep.salivarius*	–
L.bulgaricus	*B.longum*	–	–
L.plantarum	*B.lactis*	–	–
L.casei	*B.infantis*	–	–
L. GG	–	–	–
L. fermentum	–	–	–
L.reuteri	–	–	–

3. DIETARY SOURCE OF CALCIUM

Calcium-rich foods include the milk and dairy products, hard cheese, yogurt, cottage cheese, vegetables including broccoli, Chinese cabbage, green leafy vegetables like kale, mustard, collards, sardines with bones (canned), dried fruit, nuts and seeds (figs, almonds, soy nuts), and pulses (peas, beans, and lentils). A number of calcium-fortified foods and drinks are also now available *etc* [7 - 9]. Milk and dairy products have provided about 70% source of calcium, 16% from green vegetables and fruits, and 6–7% of the calcium requirement full filled with drinking water including mineral water [10].

4. PHENOMENA OF INTESTINAL CALCIUM ABSORPTION

Calcium bioavailability not only depends on source of calcium, but the age, transit time, the amount of calcium ingested, intestinal content, and type of diet also play an important role. Calcium is a divalent cation which occurs as salt in food. Calcium gets absorbed in soluble form and must be in an ionized form. As shown in Fig. (**1**) the Calcium gets absorbed *via* two transport mechanism a) transcellular and b) paracellular pathway [11]. A) Generally, transcellular pathway also termed as saturable pathway, it is a multi-step process, in which ionized calcium transport from the lumen to enterocytes across the microvillar membrane through cytosol, followed by active transport from the enterocyte to lamina propria and reaches to systemic circulation. Transcellular pathway facilitates by cytosolic calcium binding protein which is calbindin D9K. The synthesis of calbindin D9K depends upon vitamin D, hence this pathway of calcium absorption also termed as vitamin D mediates transport mechanism [12] (Table **2**). Evidence suggests that the (CaT1) cloned calcium transport protein has the ability to transport the calcium across the membrane [13]. Each stepis involved in the transcellular transport of calcium is Vitamin D dependent. B) Paracellular transport mechanism of calcium absorption involves passive transport through the tight junctions between mucosal cells. Paracellular pathway is non- saturable and independent; it is a concentration dependent pathway [13]. Most of the calcium absorption in humans occurs *via* small the intestine (duodenum, proximal jejunum, distal ileum, and some of the at colon part of GIT) [14]. About 10% of total calcium absorption occurs at the colonic region of GIT [5]. Research has been shown that the consumption of probiotics enhances the colonic calcium uptake by colonic fermentation. Mainly when larger amounts of dietary calcium are available at the site of absorption the paracellular pathway for absorption of calcium takes place

and if the calcium available at the site of absorption is in a smaller amount, the calcium gets absorbed *via* saturable pathway. The hormone-mediated up or down a plasma calcium level regulated *via* calbindin D9K I mucosal cell [15, 16].

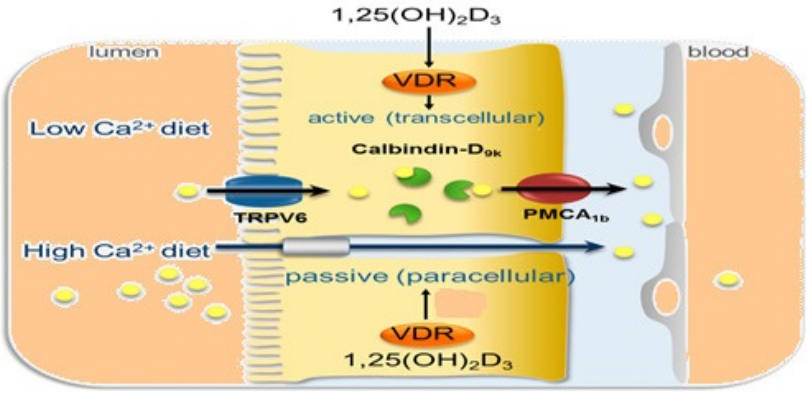

Fig. (1). Intestinal Calcium Absorption [17].

Table 2. Commercially used probiotics *Lactobacilli* and *Bifidobacteria* [12, 77].

Strain	Country	Company
Lactobacillus rhamnosus GG	Finland	Valio Dairy, Helsinki
Lactobacillus johnsonii Lal	Switzerland	Nestle, Lausanne
Lactobacillus casei Shirota	Japan	Yakult, Tokyo
Lactobacillus acidophilus NCFM	USA	Rhodia, Madison
L. casei CRL-43i Gilliland (La-Mo)	USA	Chr. Hansens, Wisconsin
Lactobacillus reuteri SD 2112	USA	BioGaia, North Carolina
Lactobacillus plantarum 299V	Sweden	Probi, Lund
L. rhamnosus 271 and *L. casei* DN 014001	France	Danone
Lactobacillus delbruekii subsp bulgaricus 2038 and *Streptococcus thermophilus* 1131	Japan	Meiji milk products, Tokyo
Lactobacillus acidophilus SBT-2062 and *Bifidobacterium longum* SBT-2928	Japan	Snow brand milk
Saccharomyces boulardii	USA	Products, Tokyo
B. longum BB536	Japan	Biocodex, Seattle
Bifidobacterium breve Yakult	Japan	Morinaga milk industry Yakult, Tokyo

5. FACTORS INHIBITING CALCIUM ABSORPTION

A diet containing phytate and oxalate forms insoluble salts with calcium and resists the calcium absorption. For the optimal calcium absorption the ideal ratio of calcium and phosphate is 1:2 and 2:1 but higher content of dietary phosphate results in the formation of insoluble calcium phosphate and prevent the calcium uptake. Impaired fat absorption leads to reaction between free fatty acids and calcium and form an insoluble calcium soap which alters the calcium absorption. Higher pH (alkaline condition) is unfavorable for the calcium absorption. Higher content of dietary fibers interferes with the calcium absorption [5, 18].

6. HOW THE PROBIOTICS SPIKES THE BIOAVAILABILITY OF CALCIUM

As we know it is difficult to get adequate calcium in daily food intakes. While, many of the factors affect the bioavailability of calcium that is available in the daily diet, for this reason it should be taken as supplement to confer the bone health and prevention of bone disease, also the lake of alertness about the daily need of nutrients become one of the major factors that impacts on bone health. As per many of the researches the two diseases, Sarcopenia (muscle loss) and Osteoporosis (bone mass loss) are responsible for aging; these two conditions majorly affect the people's good look as they age. If people consider sarcopenia and osteoporosis as serious conditions then this will help them in the right direction to live longer and lead a healthy life [19]. Many factors influence the bioavailability of Calcium in humans. Generally, it can be divided into two groups, one of it is exogenous, and another one is endogenous. Exogenous factors

are directly connected with food, and endogenous factors are related to the organism. Mainly the properties of a ingested Calcium, type and amount, oxidation status, solubility, presence of antagonistic (competitive) ions, presence of substances facilitating or hindering absorption of the minerals are the most influencing exogenous factors,while the genetic conditions, age, sex, physiological status (*e.g.* Pregnancy, breastfeeding), mineral supply status (stock), emotional state, and illnesses *etc*. are the endogenous factors [20]. As per various researches, the probiotics confer the bone health by increasing the Calcium Bioavailability, let's know how probiotics increase the calcium bioavailability and ensure the bone health. The probiotics produce short chain fatty acids, which are increasing the solubility of available calcium, simultaneously decreases the para-thyroid hormone level (increased PTH level causes the Bone resorption by stimulating the osteoclasts) and minimizes the bone loss [21 - 22]. Cereal based diets are the richest source of calcium, but concern is that the available calcium is depressed by phytate. Probiotics are producing an enzyme which is phytaze and it actively releases the depressed calcium and increase the availability of calcium at the site of absorption [24, 25]. Probiotics like Lactobacillus and bifidobacteria increase the bioavailability of calcium in some foods with estrogenic activity *via* hydrolysis of glycoside bonds of estrogenic food in the intestines [26]. As we know the vitamins play an important role in regulating the intestinal metabolism and absorption, folate and vitamin C, D and K are associated with the Calcium absorption, Probiotics are associated with the synthesis of vitamins and increase the metabolism and absorption of available calcium [26, 27]. Some Probiotics produce the bioactive peptides, which are mainly depending upon the kind of probiotics to be used. Such *as Lactobacillus helveticus* produces bioactive peptide like, proline-containing peptides isoleucyl-prolyl- proline (IPP) and valyl-prolyl-proline (VPP) which may induce greater availability of Calcium [28]. Furthermore, some of the peptides produced by probiotics are not absorbed but could support the release of minerals from insoluble ion and enhance the calcium absorption. Proline-containing peptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) may also act through preventing the formation of Angiotensin II (Ang II) from Angiotensin I (AngI), many of invitro studies had shown that the Angiotensin II stimulates the bone resorption and may also act as a vasoconstrictor in bone vasculature and increases the bone loss [27 - 29]. Researches showed that the Oxidative stress stimulates osteoclast differentiation and leads to bone resorption [30] probiotics like *Bifidobacterium longum* isolated from fermented broccoli can improve the periodontal antioxidant status by decreasing NF-kB gene expression and reduces the oxidative stress and the bone loss, ensuring the bone health. Studies have shown that adequate intake of probiotics leads to reduce intestinal inflammation and increase the bone mass density and bone mineral concentration, *L.reuteri 6475* probiotic strain significantly reduced the pro inflammatory cytokine (TNFα, IL-1β) level in jejunum and ileum of intestine, which resulted in increase in BMD, by increasing the calcium bioavailability across the intestinal mucosa [21, 31, 32]. We can say that the microbes in the gut have a significant effect on bone health [33]. Severe GIT disorders results due to intestinal mucosal damage which causes the reduction of mucosal permeability and alters the calcium absorption, but many of the studies have proved that the probiotics are having role in cell proliferation, hence an adequate intake of probiotic results in significant effect on bone health [34 - 37]. In such a way, the Probiotics are strengthening the bone and reduces the risk associated with bone loss and bone diseases *via* microbial production of metabolites or enzymes or synthesis of vitamins which are involved in calcium metabolism and are required for bone matrix formation [38 - 41].

7. STUDIES PROVES PROBIOTICS BOOSTS THE BONE HEALTH

The effect of well characterized probiotics; *Lactobacillus salivarius* (UCC 118) and *Bifidobacterium infantis* (UCC 35624)) on calcium uptake and transepithelial calcium transport in human intestinal- like, Caco-2, cells in culture and the result has shown that the Calcium transport was significantly increased by UCC 118 and *E. coli*, respectively, relative to the control. The researcher concluded that the selected strains are capable to enhance intestinal calcium uptake, if not calcium transport [42]. The investigation of the level of calcium, magnesium, phosphorus and zinc in a total of 27 samples of commercially available goat- and cow-milk fermented products and 9 samples of a goat-milk fermented product with probiotic bacterial strain and formulated the *Lactobacillus fermentum D3*. The results showedthat the bioavailability of minerals, including calcium, phosphorus, and zinc was significantly higher in the fermented milk containing the probiotic bacterial strain than it was in commercially available goat and cow milk fermented products. The researcher concluded *in-vitro* assays, strain D3 seems to increase the bioavailability of minerals compared to other milk fermented products available in the market [43]. As per one of the researches, the activity of phytases enzymes over the phytate causes complexing with minerals, Phytase enzymes are capable of sequentially dephosphorylating phytic acid to products of lower chelating capacity and higher solubility, abolishing its inhibitory effect on intestinal mineral absorption. As per the researcher, the probiotics like bifidobacteria in *Lactobacillus casei* were made by genetic construction for expressing two phytases under the control of a nisin-

inducible promoter, *L. casei* was able to produce, export and anchor the cell wall the phytase of *Bifidobacterium pseudocatenulatum*. The research showed that *Lactobacillus casei* and bifidobacteria produce enzymes having the ability to reduce the phytate content in fermented food products [44]. Many of the researches highlighted that the two diseases, Sarcopenia (muscle loss) and Osteoporosis (bone mass loss) are the major diseases responsible for aging and also that the adequate Bioavailable calcium intake overcomes these two conditions. Results of clinical trials indicating that the improvement had shown in more than thousands of patients with a chronic bone diseases, like osteoporosis and osteopenia within the six months duration of treatment [33]. Investigation about the Bioavailability of Ca, P and Zn in Rats Fed Yoghurt and Soy-yoghurt Containing Bifidobacteria in that the researcher mentioned that the Bioavailability aspects of calcium, phosphorus and Zinc from probiotic dairy foods and other food sources have been an important issue of studies over the recent years. The researcher designed a study to assess and to compare the therapeutic effect of milk, yoghurt and soy yoghurt containing bifidobacteria with regards to their effect on the bioavailability of Ca, P and Zn and bone mineralization in rats. As per that the eight groups of rats who fed the basal diet, cow milk, probiotic-free or probiotic- containing milk, yoghurts (*Bifidobacterium lactis Bb-12* or *Bifidobacterium longum Bb-46*), soy milk and soy-yoghurts containing only the above mentioned probiotics for 45 days. The results showed the rat's serum Ca and P contents were increased about two-fold that of control and the rat's serum Zn content was increased about 19-21 fold that of control [45]. Many of the researches reported that yogurt containing strains of *Lactobacillus reuteri, Lactobacillus gasseri* and *Lactobacillus casei,* increased calcium absorption and BMC in growing rats [32]. Many of the studies had proven that the probiotics like Lactobacillus and Bifidobacterium have a potential effect on increasing the BMD on mice and OVX rats [46, 47, 25, 30]. Probiotics, such as *Lactobacillus gasseri*, *Lactobacillus casei* and *Lactobacillus reuteri* have been reported to increase bone weigh about 35% higher and also reported a higher calcium absorption in rats compared to the control group. Research had been also shown that the effect of *lactobacillus casei 393* obtained from fermented milk, proven the significant effect by improving bone mass density in OVX rats which are having a lower BMD [48]. One of the studies investigated the effect of *L. paracasei* and *L. plantarum* containing fermented milk on OVX mice, the result showed that the OVX mice fed with *L.paracasei* and *L.plantarum* had a higher trabecular number compared to OVX and SOVX (sham- ovariectomized) mice as control groups [25]. The researcher examined the effect of probiotics like licheniformis and subtilis on 25 broiler chicks of one year old and divided them into two groups from which one is treatment group and another one is a control group, the result showed that no significant difference of weight and feed consumption in both the groups, but the increase in prosperous concentration and tibia ash was found in the group which fed with probiotics compared to group on normal diet [49]. One of extensive studies includes 265 postmenopausal women volunteer with age ranged from 40-87 years to examine the effect of age on calcium absorption; the researchers estimated the serum vitamin D3 metabolite, parathyroid hormone and other biochemical variables including serum creatinine level. Of the 265 volunteers about n=47 are about 40 to 45 years old, n=111 are between 56 to 65 years of age and n=25 are of greater than the 75 year old, The result showed that the calcium absorption was very low in the population by the age of >75, the results also reflected that the serum vit.D3 is not associated with a decline in calcium absorption, but the unresponsiveness of the small intestinal mucosa towards the absorption of calcium plays a vital role in older age of the population rather than the synthesis of vitamin D3 [18]. While different strains of LAB and Bifidobacterium shown potential effect on bone density in OVX rats and mice and also stimulates the osteoporosis conditions in postmenopausal OVX rats and mice [32, 47]. One of the comparison studies using group OVX and Sham-ovariectomized control groups by administering the *L.paracasei* (NTU-101) and *L.plantrum* (NTU-102) containing fermented milk, result showed that the trabecular number higher in OVX mice compared to sham-ovariectomized control groups [25]. BMC and BMD reported higher in male osteoporotic rats with Administration of *Lactobacillus helveticus* [50]. Various studies with *L.reuteri*, *L.gasseri* and *L.casei*, reported higher bone weight compared to control groups, Similarly, *Bifidobacterium longum* reported significant effects on bone health [51]. Many of the studies using LAB strains reported significant therapeutic effects on inflammatory bowel disease and rheumatoid arthritis in experimental mouse models [30, 52]. Investigation of the effect of *L.helveticus* containing fermented milk, carried out using double blind randomized crossover study by including the sample size of 20 postmenopausal women (age 50-78 years). A study was conducted in two parts, firstly the researcher divided two groups, one group was treated with fermented milk containing *L.helveticus* and another one was a controlled group, in the second part the researcher used juice containing peptides formed with *L. helveticus* bacteria were compared to a control juice. At the end of the study, they found that the fermented milk with *L.helveticus* had significant effect on calcium metabolism compared to control group, while juice containing peptides formed with L.helveticus showed no significance on calcium metabolism [29]. The researcher conducted the extensive study to check the effect of Probiotic strains *Lactobacillus helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5460 on Calcium and Hematological Parameters in Geriatrics with the sample size of 76 (38 males and 38 females) and the study duration

was from 2012 to 2015. The result showed, that the *Lactobacillus helveticus* MTCC 5463 increases serum calcium level, while no significant effect was reported on hematological parameters [53]. One of the researches carried out to investigate the effect of probiotic strains, *L. Paracasei* DSM13434, L. *plantarum* DSM 15312 and DSM 15313 (*L. mix*) on OVX induced bone loss using OVX mice. The results showed that the treatment with *L. Paracasei* DSM13434 and L. Mix reduces the cortical bone loss and bone resorption by reducing the cytokines, TNFa and IL-1b and increases the OPG expression which is an Osteoclastogenesis inhibitor [54]. The researcher studied the effect of *Bifidobacterium longum* with Yacon flour on bone health. In that study, they used 32 Wistar rats used as a study model, they divided them into four groups, from that one group as control, second one was treated with Yacon flour, third one was treated with diet and *B.longum* and fourth one was treated with Yacon flour and *B.longum.* The results showed that the calcium, phosphorous, magnesium content were higher in the group which treated with diet and *B.longum*, Yacon flour and *B.longum* compared to control group. Facture strength also found about 8.1% yacon flour, 8.6% diet with B. longum and 14.6% yacon flour with *B. longum* as compared to the control group. Yacon flour and *B.longum* fed group having more mineral concentration in the bones which is the most important factor for the prevention of osteoporosis [55]. One of the studies discusses the effect of fermented milk on utilization of inorganic constituents using hypocalcemic rats, results showing that the fermented milk reported the good source of calcium, and having a significant effect on hypocalcemic rats [56]. Also, studies proved the potency of yogurt to treat the osteoporosis in geriatric patients [57, 58]. Many of the animal and human studies reported that yogurt is a rich source of lactose, and probiotic with lactose reported significant effects in the treatment of osteoporosis [59 - 63]. One of the researches was carried out to check the effect of probiotic strain *Bifidobacterium longum* on bone density, bone mineral content, bone remodeling, bone structure and gene expression (Sparc and Bmp- genes) on bone loss from Ovariectomy rats. In that, the three groups are assigned for treatments which are a sham, OVX and OVX treated with 1 ml *Bifidobacterium longum* (10^8- 10^9 CFU/ml). The result showed that the OVX group treated with *B.longum* group reported higher serum osteocalcin and Osteoblasts, while lowering the serum C-terminal telopeptide, osteoclast and other bone resorption parameters which are mainly responsible for bone loss compared to other groups, also found that the bone mass density increased by gene expression (Sparc and Bmp- genes) [64]. One of the researches investigated the bioavailability of calcium from diets based on white cheese containing probiotic using 24 standardized white male Wistar rats (body weights ranging from 91-98g) rates are divided into four groups six rats in each group. The results showed that the groups receiving white cheese and probiotics reported higher calcium bioavailability compared to groups receiving control diet [65].

8. *IN-VITRO* MODELS FOR CALCIUM ABSORPTION STUDY

Calcium absorption using Franz diffusion cell; it has two compartments, one of it is Donor compartment and another is Acceptor compartment. The author separated both compartments using small intestine tissue of pig to simulate the actual gastric condition. They were using four different calcium salts including calcium carbonate, calcium fumarate, calcium citrate and calcium gluconate. The result showed that the 1 mmol/l ion gets migrated from donor compartment to acceptor compartment respectively the percentage rate of migration of calcium carbonate is 100% with respective pH 1.3, calcium fumarate 81.2% with respective pH 4.2, calcium citrate 81% with respective pH 6.2. The estimation of calcium carried out by UV visible spectrometer [66].

One of the extensive reviews, the author reviewed four models including solubility, Dialyzability, gastrointestinal model and CaCO-2 model generally used for bioaccessibility and bioavailability of calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc and vitamin $B_{6,}$ B_{12}, D and E [67].

Nowadays, many of the models are available commercially which simulate the human digestive system which allows a feasibility to conduct bioavailability of minerals and vitamins. The Netherlands organization for applied scientific research has developed a gastrointestinal model named TIM [68 - 70]. Table **3** TIM model simulates human gastrointestinal system, including parameters like churning, flow of saliva, body temperature, gastric juice- pancreatic juice including bile and enzymes, body temperature, regulation of intestinal pH and peristalsis. TIM model consists of two compartments which are namely TIM-1 and TIM-2, computer controlled compartment. TIM-1 consists of four chambers which respectively simulate the stomach, duodenum, jejunum and ileum. TIM-2 simulates the large intestine. One of the advantages of TIM model make possible to sample at any level of the gastrointestinal tract at any time of digestion [71].

One of the most promising tools in bioavailability and the uptake study of nutrients, including calcium is CaCO-2 model. CaCO-2 model contains cells, which belong to a human colonic adenocarcinoma epithelial cell line which simulates the intestinal cells upon culture using specific medium. This model needs to cells to grow s on the surface of

the plastic well or Trans well inserts to study the uptake of nutrient from apical to basolateral compartments, which also allows sampling of test nutrient transport from apical site to basolateral site [72 - 74].

Table 3. Probiotic products available in market.

Sr. No	Product Name	Strain	CFUs
1	Yakult	Lactobacillus Casei Shirota	6.5 Billion per unit
2	Good Belly	Lactobacillus plantrum (Lp299v)	20 Billion per 8 oz serving
3	Dan Active	Lactobacillus Casei	10 Billion per 93 ml
4	Kyo –Dophilus powder/capsules	L.gasseri, Bifidobacterium bifidum, B.longum,	1.5 Billion per serving
6	Cocobiotic	L.acidophilus, L.dulbreukii, Saccharomyces boulardii, S.cervisiae,	4 Billion
7	Nestle Actiplus Probiotic dahi	L.acidophilus	-

CONCLUSION

Present knowledge gives an idea about the major role of calcium in the prevention of bone diseases and ensuring a healthy life in all age groups of the population. Many of researches clearly indicated the involvement of probiotics in human health by acting through different mechanism. Studies are also proving the role of probiotics in calcium bioavailability. When we are talking about calcium bioavailability, it is the major challenge till now to be overcome; because of poor bioavailability of present marketed calcium formulation which are failing to fulfill the daily need of calcium. We can overcome such a problem associated with calcium bioavailability by fortifying the nutritional supplements with specific probiotic strains which could ensure 100% bioavailability of nutrients along with the calcium. The need to identify the strains which specifically act on calcium bioavailability with long term stability on fortification can be also made available in the form of cost effective stable powder formulations of such a selective strain which can be fortified with any type of drink, food and food products for maximum absorption of nutrient that are already present. Such a formulation can ensure the general health, including bone health to those populations who cannot afford costly medicines and nutrient supplements.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare that they had no conflict of interest.

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REFERENCES

[1] Brothwell D, Brothwell P. Food in antiquity: A survey of the diet of early peoples. Baltimore: Johns Hopkins University Press 1997.

[2] Nord CE, Lidbeck A, Orrhage K, Sjostedt S. Effect of supplements with *bifidobacterium longum* and *lactobacillus acidophilus* on the intestinal microbiota during administration of clindamycin. Microb Ecol Health Dis 7: 17-22.

[3] Gosbach SL. Probiotics in the third millenium digest liver dis 2002; 34(Suppl. 2): S2-7. [http://dx.doi.org/10.1016/S1590-8658(02)80155-4]

[4] Parvez1 S, Malik KA, Kang S Ah, Kim H-Y. Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 2006; 1171-85.

[5] Satyanarayana U. Text book of Biochemistry Third edition, Books & Allied (P) – Ltd, Kolkata, India, 404-407 and 123-128.

[6] Beto JA. The role of calcium in human aging. Clin Nutr Res 2015; 4(1): 1-8. [http://dx.doi.org/10.7762/cnr.2015.4.1.1] [PMID: 25713787]

[7] Dietary guidelines for Indians, national institute of nutrition Hyderabad – 500 007, India Available from: http://ninindia.org/dietaryguidelines forninwebsite.pdf

[8] USDA national nutritional database Available from: http://www.nal.usda.gov/fnic/ foodcomp/Data/ SR17/wtrank/sr17w301.pdf

[9] Calcium dietary supplement fact sheet Available from: supplements.info.nih.gov/ factsheets/calcium.asp

[10] Guéguen L, Pointillart A. The bioavailability of dietary calcium. J Am Coll Nutr 2000; 19(2)(Suppl.): 119S-36S.
[http://dx.doi.org/10.1080/07315724.2000.10718083] [PMID: 10759138]

[11] Fullmer CS. Intestinal calcium absorption: Calcium entry. J Nutr 1992; 122(3)(Suppl.): 644-50.
[http://dx.doi.org/10.1093/jn/122.suppl_3.644] [PMID: 1542026]

[12] Peng JB, Chen XZ, Berger UV, *et al.* Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J Biol Chem 1999; 274(32): 22739-46.
[http://dx.doi.org/10.1074/jbc.274.32.22739] [PMID: 10428857]

[13] Barger-Lux MJ, Heaney RP, Recker RR. Time course of calcium absorption in humans: Evidence for a colonic component. Calcif Tissue Int 1989; 44(5): 308-11.
[http://dx.doi.org/10.1007/BF02556309] [PMID: 2496901]

[14] Younes H, Demigné C, Rémésy C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. Br J Nutr 1996; 75(2): 301-14.
[http://dx.doi.org/10.1079/BJN19960132] [PMID: 8785206]

[15] Fullmer CS. Intestinal calcium absorption: Calcium entry. J Nutr 1992; 122(3)(Suppl.): 644-50.
[http://dx.doi.org/10.1093/jn/122.suppl_3.644] [PMID: 1542026]

[16] Pérez AV, Picotto G, Carpentieri AR, Rivoira MA, Peralta López ME, Tolosa de Talamoni NG. Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway. Digestion 2008; 77(1): 22-34.
[http://dx.doi.org/10.1159/000116623] [PMID: 18277073]

[17] Bronner F. Intestinal calcium absorption: Mechanisms and applications. J Nutr 1987; 117(8): 1347-52.
[http://dx.doi.org/10.1093/jn/117.8.1347] [PMID: 3305814]

[18] Nordin BE, Need AG, Morris HA, O'Loughlin PD, Horowitz M. Effect of age on calcium absorption in postmenopausal women. Am J Clin Nutr 2004; 80(4): 998-1002.
[http://dx.doi.org/10.1093/ajcn/80.4.998] [PMID: 15447911]

[19] Katharina E. Prebiotics, probiotics, and symbiotic affect mineral absorption, bone mineral content, and bone structure. J Nutr 2007; 137: 838-46.
[http://dx.doi.org/10.1093/jn/137.3.838S]

[20] Klobukowskil Jan A , Krystyna A Skibniewska, Kowalski Ireneusz M. Calcium bioavailability from dairy products and its release from food by *in vitro* digestion. J Elem 2014; 277-88.
[http://dx.doi.org/10.5601/jelem.2014.19.1.436]

[21] Campbell J, George Fahey C, Bryan A Wolf. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J Nutr 1997; 127: 130-6.

[22] Parvaneh Kolsoom, Jamaluddin Rosita, Karimi Golgis, Erfani Reza. Effect of probiotics supplementation on bone mineral content and bone mass density hindawi publishing corporation. Scientific world journal 2014; 127: 1-6.

[23] Lopez HW, Coudray C, Levrat-Verny MA, Feillet-Coudray C, Demigné C, Rémésy C. *Fructooligosaccharides* enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. J Nutr Biochem 2000; 11(10): 500-8.
[http://dx.doi.org/10.1016/S0955-2863(00)00109-1] [PMID: 11120448]

[24] Lan GQ, Abdullah N, Jalaludin S, Ho YW. Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diets. Poult Sci 2002; 81(10): 1522-32.
[http://dx.doi.org/10.1093/ps/81.10.1522] [PMID: 12412919]

[25] Chiang SS, Pan T-M. Antiosteoporotic effects of *Lactobacillus* -fermented soy skim milk on bone mineral density and the microstructure of femoral bone in ovariectomized mice. J Agric Food Chem 2011; 59(14): 7734-42.
[http://dx.doi.org/10.1021/jf2013716] [PMID: 21668014]

[26] Villa ML, Marcus R, Ramírez Delay R, Kelsey JL. Factors contributing to skeletal health of postmenopausal mexican-american women. J Bone Miner Res 1995; 10(8): 1233-42.
[http://dx.doi.org/10.1002/jbmr.5650100814] [PMID: 8585428]

[27] Weber P. The role of vitamins in the prevention of osteoporosis-a brief status report. Int J Vitam Nutr Res 1999; 69(3): 194-7.
[http://dx.doi.org/10.1024/0300-9831.69.3.194] [PMID: 10389027]

[28] Matar C, Amiot J, Savoie L, Goulet J. The effect of milk fermentation by *lactobacillus helveticus* on the release of peptides during *in vitro* digestion. J Dairy Sci 1996; 79(6): 971-9.
[http://dx.doi.org/10.3168/jds.S0022-0302(96)76448-2] [PMID: 8827460]

[29] Ma YF, Stimpel M, Liang H, Pun S, Jee WSS. Impact of antihypertensive therapy on the skeleton: Effects of moexipril and hydrochlorothiazide on osteopenia in spontaneously hypertensive ovariectomized rats. J Endocrinol 1997; 154(3): 467-74.
[http://dx.doi.org/10.1677/joe.0.1540467] [PMID: 9379124]

[30] Narva M, Nevala R, Poussa T, Korpela R. The effect of *Lactobacillus helveticus* fermented milk on acute changes in calcium metabolism in postmenopausal women. Eur J Nutr 2004; 43(2): 61-8.
[http://dx.doi.org/10.1007/s00394-004-0441-y] [PMID: 15083312]

[31] Baek KH, Oh KW, Lee WY, *et al.* Association of oxidative stress with postmenopausal osteoporosis and the effects of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures. Calcif Tissue Int 2010; 87(3): 226-35. [http://dx.doi.org/10.1007/s00223-010-9393-9] [PMID: 20614110]

[32] McCabe LR, Irwin R, Schaefer L, Britton RA. Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. J Cell Physiol 2013; 228(8): 1793-8. [http://dx.doi.org/10.1002/jcp.24340] [PMID: 23389860]

[33] Reddy MS , Reddy RK. Review and experimental clinical study of bioavailable calcium Int J Pharm Sci Nanotech 2011; 4: 1436-45.

[34] Sjögren K, Engdahl C, Henning P, *et al.* The gut microbiota regulates bone mass in mice. J Bone Miner Res 2012; 27(6): 1357-67. [http://dx.doi.org/10.1002/jbmr.1588] [PMID: 22407806]

[35] Lin PW, Tala R Nasr, Berardinelli Andrew J, Tala R. The probiotic Lactobacillus GG may augment intestinal host defence by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. Pediatr Res 2008; 64: 511-6. [http://dx.doi.org/10.1203/PDR.0b013e3181827c0f] [PMID: 18552706]

[36] Zeng H, Wu H, Sloane V, *et al.* Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. Am J Physiol Gastrointest Liver Physiol 2006; 290(1): G96-G108. [http://dx.doi.org/10.1152/ajpgi.00273.2005] [PMID: 16179598]

[37] Guma M, Firestein GS. N-terminal kinase in inflammation and rheumatic diseases. Open Rheumatol J 2012; 6: 220-31. [http://dx.doi.org/10.2174/1874312901206010220] [PMID: 23028407]

[38] Yanagihara S, Fukuda S, Ohno H, Yamamoto N. Exposure to probiotic *lactobacillus acidophilus* L-92 modulates gene expression profiles of epithelial Caco-2 cells. J Med Food 2012; 15(6): 511-9. [http://dx.doi.org/10.1089/jmf.2012.0040] [PMID: 22510151]

[39] de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 2008; 111: 1-66. [http://dx.doi.org/10.1007/10_2008_097] [PMID: 18461293]

[40] Wang MC, Luz M, Marcus Villa R, Kelsey JL. Factors contributing to skeletal health of postmenopausal Mexican- Mmerican women. J Bone Miner Res 1997; 7: 533-8.

[41] Hancock RD, Viola R. The use of micro-organisms for L-ascorbic acid production: Current status and future perspectives. Appl Microbiol Biotechnol 2001; 56(5-6): 567-76. [http://dx.doi.org/10.1007/s002530100723] [PMID: 11601603]

[42] Gilman Jennifer , Cashman Kevin D. The effect of probiotic bacteria on transepithelial calcium transport and calcium uptake in human intestinal-like caco-2 Cells Horizon Scientific Press 2006; 7(5-6): 1-6.

[43] Bergillos-Meca T, Navarro-Alarcón M, Cabrera-Vique C, *et al.* The probiotic bacterial strain *Lactobacillus fermentum* D3 increases *in vitro* the bioavailability of Ca, P, and Zn in fermented goat milk. Biol Trace Elem Res 2013; 151(2): 307-14. [http://dx.doi.org/10.1007/s12011-012-9544-0] [PMID: 23179347]

[44] Yebra Maria J, Haros Monika, Monedero Vicente. IzaskunGarcía-Mantrana. Expression of bifidobacterial phytase in *L.casei* and their application in a food model of whole-grain sourdough bread. Int J Food Microbiol 2016; 216: 18-24. [http://dx.doi.org/10.1016/j.ijfoodmicro.2015.09.003] [PMID: 26384212]

[45] Abd El-Gawad IA, Mehriz Abou Elsamh M, Saleh Farag A, *et al.* Bioavailability of Ca, P and Zn and bone mineralization in rats fed yoghurt and soy- yoghurt containing bifidobacteria. Eur J Nutr Food Saf 2014; 4(2): 110-26. [http://dx.doi.org/10.9734/EJNFS/2014/6856]

[46] Tomofuji T, Ekuni D, Azuma T, *et al.* Supplementation of broccoli or bifidobacterium longum-fermented broccoli suppresses serum lipid peroxidation and osteoclast differentiation on alveolar bone surface in rats fed a high-cholesterol diet. Nutr Res 2012; 32(4): 301-7. [http://dx.doi.org/10.1016/j.nutres.2012.03.006] [PMID: 22575044]

[47] Ghanem KZ, Badawy IH. Influence of yoghurt and probiotic yoghurt on the absorption of calcium, magnesium, iron and bone mineralization in rats. Milchwissenschaft 2004; 59: 472-5.

[48] Effects of a lactobacillus casei 393 fermented milk product on bone metabolism in ovariectomized rats. Int Dairy J 2009; 19: 690-5. [http://dx.doi.org/10.1016/j.idairyj.2009.06.009]

[49] Mutuş R, Kocabagli N, Alp M, Acar N, Eren M, Gezen SS. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. Poult Sci 2006; 85(9): 1621-5. [PMID: 16977848]

[50] Kwon H-K, Lee C-G, So J-S, *et al.* Generation of regulatory dendritic cells and $CD4^{+}Foxp3^{+}$ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci USA 2010; 107(5): 2159-64. [http://dx.doi.org/10.1073/pnas.0904055107] [PMID: 20080669]

[51] Yousf Hamid, Tomar Geetanjali B, Srivastava Rupesh K, *et al.* Probiotics and bone health: It takes GUTS to improve bone density. Int J Immunother Cancer Res 2015; 1(1): 018-22.

[52] Ghanem KZ, *et al.* Influence of yoghurt and probiotic yoghurt on the absorption of calcium, magnesium, iron, and bone mineralization in rats. Milchwissenschaft 2004; 59: 472-5.

[53] Gohel Manisha Kalpesh, Prajapati Jashbhai B, Mudgal Sreeja V, *et al.* Effect of probiotic dietary intervention on calcium and haematological parameters in geriatrics. J Clin Diagn Res 2016; 10(4): 5-9. Apr

[54] Ohlsson C, Engdahl C, Fåk F, *et al.* Probiotics protect mice from ovariectomy-induced cortical bone loss. PLoS One 2014; 9(3): e92368. [http://dx.doi.org/10.1371/journal.pone.0092368] [PMID: 24637895]

[55] Rodrigues FC, Castro AS, Rodrigues VC, *et al.* Yacon flour and bifidobacterium longum modulate bone health in rats. J Med Food 2012; 15(7): 664-70. [http://dx.doi.org/10.1089/jmf.2011.0296] [PMID: 22510044]

[56] Depuis Y. Fermented milk and utilization of inorganic constituents Ann. Bull Int Dairy Fed 1964; 3: 36-43.

[57] Seneca H, Gaymont S. Clinical uses of yogurt. J Am Geriatr Soc 1957; 5(11): 932-5. [http://dx.doi.org/10.1111/j.1532-5415.1957.tb00492.x] [PMID: 13480816]

[58] Bushnell PJ, DeLuca HF. Lactose facilitates the intestinal absorption of lead in weanling rats. Science 1981; 211(4477): 61-3. [http://dx.doi.org/10.1126/science.7444448] [PMID: 7444448]

[59] Cochet B, Jung A, Griessen M, Bartholdi P, Schaller P, Donath A. Effects of lactose on intestinal calcium absorption in normal and lactase-deficient subjects. Gastroenterology 1983; 84(5 Pt 1): 935-40. [PMID: 6403404]

[60] Sato R, Noguchi T, Naito H. Effect of lactose on calcium absorption from the rat small intestine with a non-flushed ligated loop. J Nutr Sci Vitaminol (Tokyo) 1983; 29(3): 365-73. [http://dx.doi.org/10.3177/jnsv.29.365] [PMID: 6619997]

[61] Savaiano DA, Levitt MD. Nutritional and therapeutic aspects of fermented dairy products. ASDC J Dent Child 1984; 51(4): 305-8. [PMID: 6590586]

[62] Smith TM, Kolars JC, Savaiano DA, Levitt MD. Absorption of calcium from milk and yogurt. Am J Clin Nutr 1985; 42(6): 1197-200. [http://dx.doi.org/10.1093/ajcn/42.6.1197] [PMID: 3934956]

[63] Newcomer AD, Hodgson SF, McGill DB, Thomas PJ. Lactase deficiency: Prevalence in osteoporosis. Ann Intern Med 1978; 89(2): 218-20. [http://dx.doi.org/10.7326/0003-4819-89-2-218] [PMID: 677585]

[64] Parvaneh K, Ebrahimi Mahdi, Sabran Mohd Redzwan. Probiotics (bifidobacterium longum) increase bone mass density and upregulate sparc and bmp-2 genes in rats with bone loss resulting from ovariectomy. Hindawi publishing corporation bio med research international volume 2015; pp. 1-10.

[65] Klobukowski Jan. Calcium bioavailability from diets based on white cheese containing probiotics or synbiotics in short-time study in rats. Pak J Nutr 2009; 8(7): 933-6. [http://dx.doi.org/10.3923/pjn.2009.933.936]

[66] Dolinska B, Mikulska A, Caban A, Ostrozka-Cieslik A, Ryszka F. A model for calcium permeation into small intestine. Biol Trace Elem Res 2011; 142(3): 456-64. [http://dx.doi.org/10.1007/s12011-010-8827-6] [PMID: 20809269]

[67] Etcheverry P, Grusak MA, Lisa E. Fleige application of *in-vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc and vitamins B_6, B_{12}. D and E 2012; 3(317): 1-22.

[68] Afkhami F, Ouyang W, Chen H, Lawuyi B, Lim T, Prakash S. Impact of orally administered microcapsules on gastrointestinal microbial flora: In-vitro investigation using computer controlled dynamic human gastrointestinal model. Artif Cells Blood Substit Immobil Biotechnol 2007; 35(4): 359-75. [http://dx.doi.org/10.1080/10731190701460226] [PMID: 17701483]

[69] de Jong P, Vissers MM, van der Meer R, Bovee-Oudenhoven IM. *In silico* model as a tool for interpretation of intestinal infection studies. Appl Environ Microbiol 2007; 73(2): 508-15. [http://dx.doi.org/10.1128/AEM.01299-06] [PMID: 17122404]

[70] Minekus M, Marteau P, Havenaar R, Huis in't, Veld J H J. A multi compartmental dynamic computer-controlled model simulating the stomach and small intestine. ATLA 1995; 23(2): 197-209.

[71] Etienne-Mesmin L, Livrelli V, Privat M, *et al.* Effect of a new probiotic *saccharomyces cerevisiae* strain on survival of *Escherichia coli* O157:H7 in a dynamic gastrointestinal model. Appl Environ Microbiol 2011; 77(3): 1127-31. [http://dx.doi.org/10.1128/AEM.02130-10] [PMID: 21131521]

[72] Gangloff MB, Lai C, Van Campen DR, Miller DD, Norvell WA, Glahn RP. Ferrous iron uptake but not transfer is down-regulated in caco-2 cells grown in high iron serum-free medium. J Nutr 1996; 126(12): 3118-27. [http://dx.doi.org/10.1093/jn/126.12.3118] [PMID: 9001382]

[73] Protocol for CaCO-2 model online.org/prot/Protocols/ Protocol-for-Caco-2-cell-culture-4454.html

[74] Galán I, García ML, Selgas MD. Effects of irradiation on hamburgers enriched with folic acid. Meat Sci 2010; 84(3): 437-43. [http://dx.doi.org/10.1016/j.meatsci.2009.09.013] [PMID: 20374807]

[75] Emerging trends of probiotics in formulation development as a biotherapeutics agent. AJPRHC 2010; 4(1): 42-51.

[76] Fontana L, Bermudez-Brito M, Plaza-Diaz J, Muñoz-Quezada S, Gil A. Sources, isolation, characterisation and evaluation of probiotics. Br J

Nutr 2013; 109(Suppl. 2): S35-50.
[http://dx.doi.org/10.1017/S0007114512004011] [PMID: 23360880]

[77] World Gastroenterology Organisation Global Guidelines Probiotics and Prebiotics October 2011.