

## Functionality of Dairy Foods on Osteoporosis

Yong-Il Moon<sup>1</sup>, Sunho Lee<sup>2</sup>, Sangnam Oh<sup>3</sup> and Younghoon Kim<sup>3</sup>

<sup>1</sup>Dept. of Animal Source Foods, Woosuk University, Wanju, Korea

<sup>2</sup>Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul, Korea

<sup>3</sup>Dept. of Animal Science and Institute of Milk Genomics, Chonbuk National University, Jeonju, Korea

### Abstract

Osteoporosis is a systemic skeletal disease characterized by decreased bone mass, which results in a markedly increased risk of traumatic fractures. This disease is a worldwide health problem with a high prevalence. Recently, various dietary components have been found to minimize the risk of developing osteoporosis through their ability to stimulate bone formation and optimize bone health. Among them, probiotics and fermented milk can have beneficial effects to human health. Bioactive compounds derived from probiotics in fermented milk can especially modulate physiological functions related to bone health. Here, we review the evidence to support these insights into newly found functionality of dairy foods for osteoporosis prevention.

### Keywords

dairy foods, osteoporosis, probiotics, fermented milk

## Probiotics

Definitions of the term “probiotic” have been used over a few decades, but one of the recent interpretations of the word was derived by the Food and Agriculture Organization of the United Nations-World Health Organization (FAO-WHO), and it is endorsed by the International Scientific Association for Probiotics and Prebiotics. They mentioned that: “Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host”. In recent, it is getting accepted more and more by consumers which live lactic acid bacteria does exert health benefits when they get eaten. In addition, it is also becoming recognized that not all probiotic bacteria are equal (Dekker *et al.*, 2007). It should also be added that lactic acid bacteria does taking a major role in human intestine as probiotics. Since the early studies of Metchnikoff of the favorable effects of soured milk products in humans, the most beneficial part of the intestinal microbiota has been suggested to be lactic acid bacteria. Moreover, Lactic acid bacteria are treated as the easiest findable commercial probiotics preparations still in these years. For these reasons, studies of lactic acid bacteria are becoming an important part which related with many people’s health. The various probiotics group were evaluated that *Lactobacillus acidophilus*, *L. salivarius*, *L. casei*, *L. plantarum*, *L. fermentum*, *L. reuteri*, *Pediococcus acidilactici* and *Bifidobacterium* spp. (modified from Salminen *et al.*, 2004, Table 1). The many commercialized probiotics strains have been identified and useful for dairy products such as fermented milk in the market (modified from Yeung *et al.*, 1999, Table 2).

The probiotic products traditionally incorporate intestinal species of *Lactobacillus* because of their long history of safe use in the dairy industry and their natural presence in the human

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\*Corresponding author :  
Younghoon Kim, Dept. of Animal  
Science and Institute of Milk  
Genomics, Chonbuk National  
University, Jeonju, Korea. Tel :  
+82-63-270-2606, Fax :  
+82-62-270-2612, E-mail :  
ykeys2584@jbnu.ac.kr

**Table 1.** Microorganisms usually connected with beneficial probiotic and dietary properties in cultured milks

Probiotics	Strains
<i>Bifidobacterium</i> species	<i>Bifidobacterium longum</i> <i>Bifidobacterium bifidum</i> <i>Bifidobacterium infantis</i> <i>Bifidobacterium animalis</i> <i>Bifidobacterium lactis</i>
<i>Pediococcus</i> species	<i>Pediococcus acidilactici</i>
<i>Lactobacillus</i> species	<i>Lactobacillus delbruekii</i> supsp. <i>bugaricus</i> <i>Lactobacillus acidophilus</i> <i>Lactobacillus casei</i> <i>Lactobacillus paracasei</i> <i>Lactobacillus plantarum</i> <i>Lactobacillus reuteri</i> <i>Lactobacillus rhamnosus</i>
<i>Propionibacterium</i> species	<i>Propionibacterium freudenreichii</i>
<i>Enterococcus</i> species	<i>Enterococcus faecium</i> (previous name <i>Streptococcus faecium</i> )

intestinal tract, which is known to contain a myriad of microbes, collectively called the microbiota (Tannock, 1998; Falk *et al.*, 1998). Members of the *Bifidobacterium* have more recently been added to foods for probiotic purposes, probably encouraged by the discovery of their consistent presence as part of the normal microbiota of the human intestine (Tannock, 1999). The desirable effects on human health include antagonistic activity against pathogens, anti-allergic effects and other effects on the immune system. As evidence accumulates for their beneficial effects on human health, these bacteria are increasingly being included as functional ingredients, particularly in dairy products such as yoghurts and other fermented milks (Fig. 1). Nevertheless, there are some reasonable skeptical opinions among the scientific community about probiotic effects; because, the mechanistic details which underlying probiotic effects are just simply unknown. Although the effects of the gut commensal microbiota are necessary for development and maintenance of normal

**Table 2.** Characterized of commercial probiotic strains

Strain	Source
<i>Lactobacillus acidophilus</i> NCFM	Rhodia, Inc. (Madison, Wis.)
<i>Lactobacillus acidophilus</i> DDS-1	Nebraska Cultures, Inc. (Lincoln, Neb.)
<i>Lactobacillus acidophilus</i> SBT-2062	Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)
<i>Lactobacillus acidophilus</i> LA-1	Chr. Hansen, Inc. (Milwaukee, Wis.) (same as strain LA-5 sole in Europe)
<i>Lactobacillus casei</i> Shirota	Yakult (Tokyo, Japan)
<i>Lactobacillus casei</i> Immunitas	Danone (Paris, France)
<i>Lactobacillus fermentum</i> RC-14	Urex Biotech (London, Ontario, Canada)
<i>Lactobacillus johnsonii</i> La1	Nestle' (Lausanne, Switzerland) (same as Lj1)
<i>Lactobacillus paracasei</i> CRL 431	Chr. Hansen, Inc. (Milwaukee, Wis.)
<i>Lactobacillus plantarum</i> 299V	Probi AB (Lund, Sweden) (same as MM2)
<i>Lactobacillus reuteri</i> SD2112	Biogaia (Raleigh, N.C.)
<i>Lactobacillus thamnusus</i> GG	Valio Dairy (Helsinki, Finland)
<i>Lactobacillus thamnusus</i> GR-1	Urex Biotech (London, Ontario, Canada)
<i>Lactobacillus thamnusus</i> 271	Probi AB (Lund, Sweden)
<i>Lactobacillus rhamnosus</i> LB21	Essum AB (Umea, Sweden)
<i>Lactobacillus salivarius</i> UCC118	University College (Cork, Ireland)
<i>Lactobacillus lactis</i> L1A	Essum AB (Umea, Sweden)
<i>Bifidobacterium lactis</i> Bb-12	Chr. Hansen, Inc. (Milwaukee, Wis.)
<i>Bifidobacterium longum</i> BB536	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
<i>Bifidobacterium longum</i> SBT-2928	Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)
<i>Bifidobacterium breve</i> strain Yakult	Yakult (Tokyo, Japan)

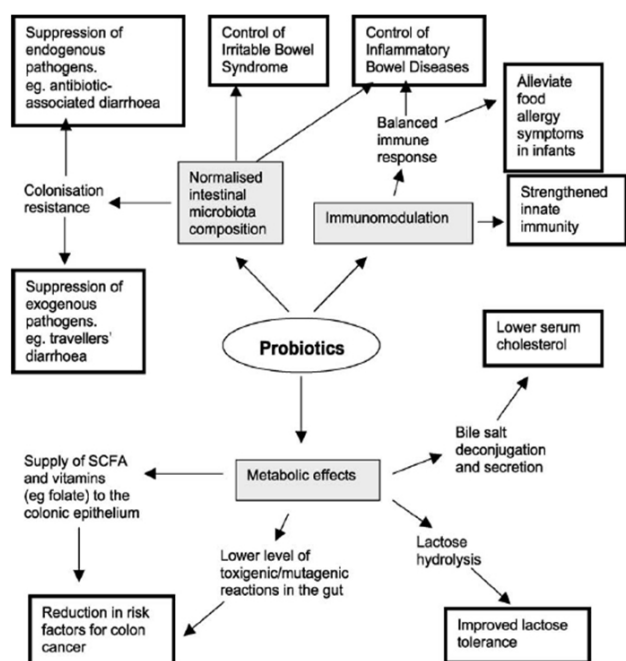


Fig. 1. Proposed health benefits stemming from probiotic consumption (adapted from Saarela *et al.*, 2002)

health, the effects of individual members on our health are still not understood even though the fact has been recognized for decades.

## Fermented Milk

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals until they are able to digest other types of foods. Early-lactation milk contains colostrum, which carries the mother's antibodies to the baby, can prevent a baby from the risk of many diseases. Milk is an important food with many nutrients (Pehrsson *et al.*, 2000). Milk contains nutrients which required for the growth and development of the neonate. All milks contain specific proteins and fats which designed to make digest easier, and also the milks contains lactose, minerals, vitamins, and other components which taking an important roles. These are organized as follows: lipids in emulsified globules coated with a membrane, proteins in colloidal dispersion as micelles, and most minerals and all lactose in true solution (Jensen *et al.*, 1991). In many cultures of the world, humans continue to drink milk from other

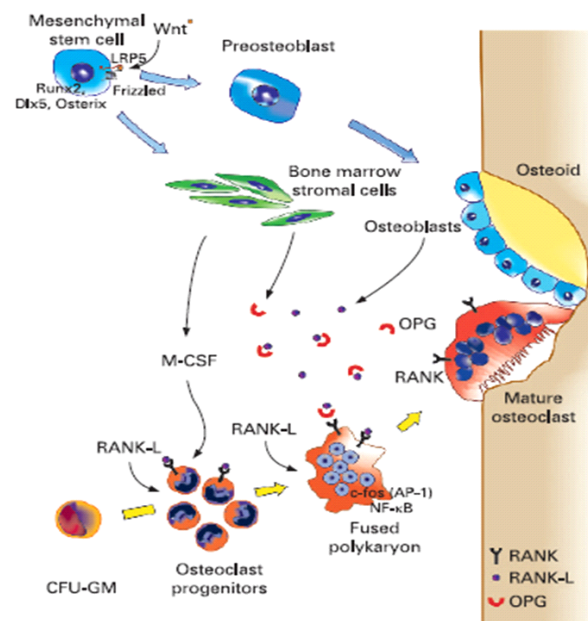
animals (especially cattle, goats and sheep) as a dairy foods. Among them, fermented milk products, also known as cultured dairy foods, cultured dairy products, or cultured milk products, are dairy foods that have been fermented with lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc*. The fermentation process increases the shelf-life of the product, as well as adds to the taste and improves the digestibility of milk. While numerous researchers (Taranto *et al.*, 1998; Lee *et al.*, 1999; Danone, 2001) have suggested that lactic culture and their fermented products provide several nutritional and therapeutic benefits to the consumers. The majority of the papers suggest that the potential benefit following the consumption of fermented dairy products containing viable lactic acid bacteria (Deeth and Tamime., 1981; Fernandes *et al.*, 1987; Gilliland, 1990; Fujiwara *et al.*, 1997; Gill and Guarner, 2004) is primarily attributable to the favorable alteration in GI micro-ecology.

## Osteoporosis

Osteoporosis defined by the working group of the World Health Organization (WHO) as a bone density T score at or < 2.5 SD below normal peak values for young adults. The osteoporosis primary definition is a skeletal disorder of post-menopausal women or of old people. But, recently definition is a systemic skeletal disease characterized by low bone mass and the micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Marcus *et al.*, 2001). Osteoporosis is mainly a primary disease. With the increase of age, it is progressive and irreversible. Also, osteoporosis can cause or be complicated with lumbodorsal pain, malignant tumors, and other diseases. Therefore, it is not only a medical concern but also a serious health care and social problem. Furthermore, given the clear sex-related difference in the incidence of this disease, such interventions should be readily undertaken for women, and especially for postmenopausal women whose bone mass rapidly decreases (Ylikorkala, 2008; Ohta *et al.*, 1999; Dane *et al.*, 2008; Al-Azzawi, 2008). Since osteoporosis is influenced by genetic and environmental factors such as diet and lifestyle, it is important to develop strategies and recommendations for both the treatment and prevention of osteoporosis. In recent years, nutrients have

received considerable interest because of their potential to prevent or minimize the risk of developing osteoporosis (Weber, 1999; Cashman, 2007). Osteoporosis could be divided into two distinct syndromes (Type I and Type II osteoporosis) that different symptoms such as in regional bone mineral density (BMD), fracture patterns, hormone changes and mechanism. Type I osteoporosis typically affects women within 15~20 years after menopause. This type characterized by fractures occurring at sites that contain relatively large amount of cancellous bone therefore increased tooth loss occurs in type I osteoporosis. And type II occurs in both sex, but is twice as common in women as in men. Type II osteoporosis is characterized vertebral fracture that hip fracture, pelvis, proximal humerus and proximal tibia (Riggs *et al.*, 2001).

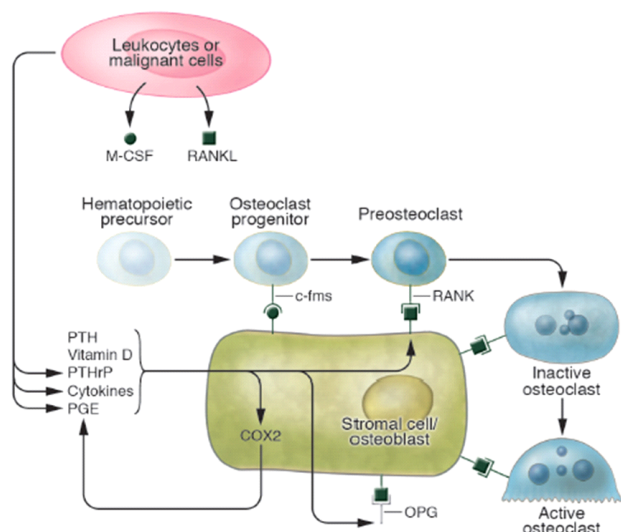
Osteoporosis disease was characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture. The bone loss results from imbalance between the activities of osteoblast and osteoclast that leads to uncoupling of bone formation and bone resorption. Bone homeostasis is maintained by a balance between bone formation by osteoblasts and bone resorption by osteoclasts, respectively (Sprague, 2000). Osteoblasts are derived from a multi-potential mesenchymal cell that can alternatively differentiate also into marrow stromal cells or adipocytes (Aubin, 2001; Silvernoinen *et al.*, 1995). The signals that are involved for the development of osteoblasts from mesenchymal progenitor cells are not fully understood (Walsh *et al.*, 2006; Frei *et al.*, 2006). Wnt signalling through Wnt10b is involved in the fate decision in differentiating mesenchymal progenitor cells between adipocytes and osteoblasts (Bennett *et al.*, 2005). Osteoclasts are multi-nucleated macrophage-like giant cells that resorb bone (Teitelbaum, 2000). They are hematopoietic in origin and derive from myeloid precursors that also give rise to macrophages and dendritic cells. Signals that control osteoclasts to form and resorb bone involve several transcription factors and cytokines which are discussed later in detail (Raisz, 2005, Fig. 2). The critical role of nuclear factor kappa B (NFκB) in osteoclast formation has been demonstrated in NFκB1 and NFκB2 knockout mice that showed osteopetrosis caused by a defect in osteoclast development (Iotsoval *et al.*, 1997). To remove organic components of bone, osteoclasts produce several



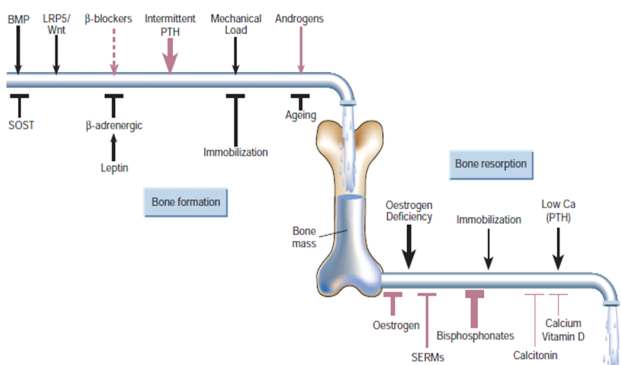
**Fig. 2.** Bone remodeling under physiological conditions (adapted from Tilg *et al.*, 2008)

enzymes such as cathepsin. Osteoclasts are highly motile, move across the bone surface, and resorb large areas of bone (Teitelbaum, 2000; Lawrence, 2005, Fig. 3). They died by apoptosis regulated by several paracrine acting cytokines. Inflammation-related osteoporosis is driven by activation of osteoclasts and therefore this cell type is of critical importance.

Bone formation and resorption are 'coupled' locally by mechanisms not fully understood, that is, when one goes up or down the other usually follows. But resorption is much faster than formation (it takes at least three months to rebuild bone resorbed in 2-3 weeks). Thus, increased resorption, even then accompanied by coupled increase formation, can cause bone loss owing to these kinetic differences; for instance, in estrogen deficiency or hyperparathyroidism. Multiple coupling factors attempt to maintain this servo system at its physiological (homeostatic) steady state (Fig. 4). Bone formation stimulatory factors released from the matrix during resorption, including insulin-like growth factor and transforming growth factor (TGF)- $\beta$ , may serve this function. Several factors, such as PTH, PGE, fibroblast growth factor, TGF- $\beta$  and even RANK ligand, have been shown to stimulate both resorption and formation. Mechanical effects on bone could also couple



**Fig. 3.** Regulation of osteoclast formation and activity (adapted from Lawrence, 2005).



**Fig. 4.** Various factors of affecting on bone formation and resorption (adapted from Harada and Rodan, 2003).

resorption to formation, as weakening of the bone as a result of resorption should engender corrective bone formation (Harada and Rodan, 2003).

Lifestyle prevention of osteoporosis is in many aspects inversions from potentially modifiable risk factors. As tobacco smoking and unsafe alcohol intake have been linked with osteoporosis, smoking cessation and moderation of alcohol intake are commonly recommended in the prevention of osteoporosis. Many other risk factors, some modifiable and others not modifiable, such as genetic, may be involved in osteoporosis (Davis *et al.*, 2010). Whether or not calcium (with or without vitamin D) is effective in preventing osteoporosis is controversial. A 2007 review supported its use for pre-

vention in people over the age of 50 years (Tang *et al.*, 2007). A review and draft recommendation by the United States Preventive Services Task Force in 2012 found insufficient evidence to determine risk versus harm from supplementation in women before menopause or in men, so does not recommend it for this group (Vitamin D and Calcium Supplementation to Prevent Cancer and Osteoporotic Fractures in Adults: U.S. Preventive Services Task Force Recommendation Statement, 2012). People at risk for osteoporosis (e.g. steroid use) are generally treated with vitamin D and calcium supplements and often with bisphosphonates. Vitamin D supplementation alone does not prevent fractures, and needs to be combined with calcium (Sahota, 2010; The DIPART group, 2010). Estrogen replacement therapy remains a good treatment for prevention of osteoporosis, but is not recommended yet unless other indications for its use are present, as well. There is uncertainty and controversy about whether estrogen should be recommended in women in the first decade after the menopause. In hypogonadal men, testosterone has been shown to give improvement in bone quantity and quality, but, as of 2008, no studies of the effects on fractures or in men with a normal testosterone level have been reported (Ebeling, 2008).

The clinically useful bisphosphonates are synthetic analogues of inorganic pyrophosphate, an endogenous regulator of bone turnover that inhibits bone resorption and mineralization *in vitro* (Fleisch, 1991). All bisphosphonates have a high affinity for hydroxyapatite but, unlike pyrophosphate, are resistant to metabolism by endogenous phosphatases (Fleisch, 1991; Schenk *et al.*, 1986). Bisphosphonates are capable of inhibiting bone mineralization at roughly equivalent doses; however, their potencies for the inhibition of bone resorption depend on the unique chemical structure of their side chains (Shinoda *et al.*, 1983). The four-carbon amino side chain of alendronate conveys a very high potency, which in turn permits effective inhibition of osteoclast-mediated bone resorption at doses that do not impair bone mineralization, even with daily doses, which is not the case with etidronate (Marcus, 1993; Axelrod and Teitelbaum, 1994). Treatment with alendronate specifically inhibits increased bone resorption and thereby normalizes the rate of bone turnover (Garnero *et al.*, 1994). Preclinical evaluations of alendronate in animals with osteoporosis have documented greater bone strength in accordance with

increased bone mass, (Guy *et al.*, 1993; Balena *et al.*, 1993) indicating the normal quality of alendronate- treated bone. Iliac-crest biopsies in patients treated with alendronate for up to three years show normal bone without evidence of mineralization defects (Arlot *et al.*, 1995).

## Dairy Products and Osteoporosis

Dairy products such as milk have been proposed as a nutritional food that aids in the prevention of osteoporosis due to its bioavailable calcium content (Silverwood, 2003). Lactoferrin (iron-binding glycoprotein) is a non-enzymatic antioxidant found in the whey fraction of milk (Marshall, 2004). Lactoferrin has functional bone metabolism that is promotes the osteoblast cell growth and reduces osteoclast differentiation and resorption activity (Cornish, 2004; Lorget *et al.*, 2002). *In vitro* and *in vivo* determined milk basic protein (MBP) has the ability to stimulate proliferation and differentiation of osteoblastic cells as well as suppress bone resorption (Takada *et al.*, 1997a; Toba *et al.*, 2000). MBP is prepared from fractionated whey through cation exchange resin. *In vivo* studies on rats determined that both whey protein and fractionated whey protein had the ability to increase femoral bone strength in ovariectomized rats (Takada *et al.*, 1997b; Kato *et al.*, 2000). Recently reports, the fermented milk by *L. helveticus* contains small peptides such as isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) (Nakamura *et al.*, 1995). This small peptides are inhibits the angiotensin converting enzyme (ACE). Fermented milk containing IPP and VPP long term fed on rats has been lowered the blood pressure (Sipola *et al.*, 2002). So this fermented milk by *L. helveticus* affects calcium accumulation on osteoblasts (Narva *et al.*, 2004). In conclusion, bioactive compounds from dairy foods including fermented milk can provide new functionality on prevention of osteoporosis.

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