CHAPTER-I

INTRODUCTION
According to the World Health Organization, osteoporosis and cardiovascular disorders, both are critical health issues worldwide and the cost of healthcare for their management is high. It is been recently have shown that, cardiovascular disease causes more than 16.7 million deaths per year throughout the world (Yach et al., 2004). On the other hand it is estimated that, in world for every three seconds fracture occurs due to osteoporosis, at 50 years of age, one in three women and one in five men will be suffering from fracture in their remaining lifetime. For women, the risk of hip fracture is higher than the risk of breast, ovarian and uterine cancer combined. For men, the risk is higher than the risk for prostate cancer.

Approximately 50% of people with one osteoporotic fracture will have another, with the risk of new fractures rising exponentially with each fracture (iofbonehealth.org/WOD/2013/thematic-report/WOD13-Report). For the treatment of osteoporosis just in the USA costs around ~$14 billion, annually (Cummings S.R 1998). Whereas, North America, as recently highlighted by the “2Million2Many” Campaign of the U.S. National Bone Health Alliance evaluated the incidence and costs of osteoporosis for the period 2005-2025 concluding the possibility of occurring around 2 million fragility fractures annually in the United States (Burge et al., 2007).

In Asia 1995, 5.3% of the population was aged 65 years and over; this is projected to increase to 9.3% by 2025, representing increase of 75% for a population of several billion people. During the year 2009, there were 167 million people aged over 60 years living in China, which will rise to 480 million by 2050 (Brangian T 2012). Almost 700 000 hip fractures occur annually in China. Alarmingly, from 2002 to 2006, hip fracture
rates among those over 50 years of age in Beijing increased by 58% in women and 49% in men (Xia et al., 2012). Major changes in urbanization and in lifestyle are proposed as the primary reasons for such a rapid change. In India, total of 36 million people were already suffering from osteoporosis. By 2050, it is predicted to have more than 50% of all osteoporotic fractures in Asia. In terms of costs, projections for China illustrate the financial burden that is looming across that region. In 2006, US$1.6 billion was spent on hip fracture care in China; this is projected to rise to US$12.5 billion by 2020 and $265 billion by 2050 (http://www.2million2many.org/).

In order to prevent this disease, studies by many researchers have shown that menaquinone-7 (MK-7) consumption substantially reduces the risk of bone fractures and cardiovascular disorders (Kaneki et al., 1995; Tamatani et al., 1998; Yamaguchi et al., 1999; Tsukamoto et al., 2000a; Tsukamoto et al., 2000b Schurgers et al., 2007; Gast et al., 2009). There is not, as yet, a recommended therapeutic dose for MK-7 according to age and sex. However, it has been suggested, 30 mg of daily intake to prevent osteoporosis and coronary/artery calcification (Morikawa et al., 2010). Menaquinone-7 (MK-7) is a highly valuable member of the vitamin K family has a significant effect on preventing osteoporosis and cardiovascular diseases besides its positive effects on blood coagulation (Yamaguchi et al., 1999; Gast et al., 2009; Schurgers et al., 2007). MK-7 is a part of the family known as Vit-K2, and is essential for the synthesis of blood coagulation factor, and it also helps in the activation of proteins which is involved in the building of bones and inhibition of vascular calcification (Bruge et al., 2011; Rheaume et al., 2011). MK-7 has shown to have an anabolic effect on bone calcification in rat femoral tissue. Zinc has been shown to enhance the effect of MK-7 in increasing bone calcium content in vitro (Ehara et al., 1996). The combined administration of zinc and MK-7 was found to have a synergistic or additive enhancing effect on bone components in the femoral tissue.
of female elderly rats (Ma et al., 2001). MK-7 may be significant in preventing osteoporosis with increasing age of person. Recently, in vitro study has been demonstrated that MK-7 can directly stimulate calcification in femoral metaphyseal tissue obtained from normal rats (Sato et al., 1996; Yamaguchi et al., 1999). MK-7 is available in food products such as meat, cheese and fermented soybeans (natto); the latter has the highest MK-7 concentration (800-900 μg/100g) (Sakano et al., 1988). Nevertheless, the concentration of MK-7 in food products is too low for therapeutic applications. The digestion and utilization of MK-7 from these sources in human bodies becomes less efficient as aging occurs (Howard and Payne, 2006). All of these have shown the need of industrial production of supplementary MK-7.

MK-7 is produced mainly by liquid state fermentation using Bacillus subtilis species (Beulens et al., 2009; Sato et al., 2001b) and solid state fermentation (Mahanama et al., 2011). Natto is a traditional fermented soybean food in Japan; it contains comparatively high amounts of MK-7 when compared with other foods (Sakano et al., 1988). Since the strains of B. subtilis used for manufacturing natto are edible, they are among the best sources of MK-7 in the food industry. To improve the production of MK-7, studies have focused on genetic mutation and the nutrients in the fermentation media. Many previous studies have been carried out largely on microorganism isolation and process development in order to enhance vitamin K2 production (Sato et al., 2001; Tsukamoto et al., 2001; Berenjian et al., 2011; Mahanama et al., 2011).

In view of the potential applications of MK-7 and need for development of economical method for improved MK-7 production with an aim of reducing the cost of industrial process, the present study was under taken. MK-7 conventionally produced by submerged fermentation by using defined media. Various microbial strains have been
explored for MK-7 production employing synthetic media. Hence this work was focused on the biotechnological production of MK-7, a vital agent in treating osteoporosis, through submerged fermentation, which is a promising and cost effective technology for the present day.

1.1 AIM OF THE PRESENT STUDY

The aim of the present study is to evaluate the potentiality of *Bacillus thuringiensis* KLSG-9 for MK-7 production under submerged fermentation.

1.2 OBJECTIVES OF THE STUDY

The present study was undertaken with the following objectives:

1) Isolation and screening of organism for vitamin K2 (Menaquinone-7) production.

2) Evaluation of fermentation methods (solid state fermentation and submerged fermentation) for the suitability of vitamin K2 (MK-7) production by the isolates.

3) Optimization studies for the production of vitamin K2 (MK-7).

4) Strain improvement programme for enhanced production of vitamin K2 (MK-7)

5) Extraction, purification and characterization of vitamin K2 (MK-7).

6) Application studies of vitamin K2 (MK-7).