The relationship between certain food and its health benefits has been investigated from many years. Development of foods that confer health benefits on the host is the key research priorities of the food industry (Klaenhammer and Kullen, 1999). These studies led to the production and consumption of foods enriched with active components such as probiotics, prebiotics and synbiotics which are recognized as functional foods. The term functional food was introduced in Japan and this type of foods is known as foods for specified health use (Shanahan, 2004). The American Dietetic Association (2005) defined functional foods as all those foods that provide health benefits beyond their nutrition. People consuming a diet rich in functional or bioactive components are at lower risk of chronic illnesses and with lower mortality rates.

Metchnikoff (1907) pointed out the use of fermented milks in the diet for prevention of certain diseases of the gastrointestinal tract and promotion of healthy day-to-day life. Since then, a number of studies have shown that the fermented food products do have a positive effect on health status in many ways (Gustafsson, 1983). A fermented food product or live microbial food supplement, which has beneficial effects on the host by improving intestinal microbial balance, is generally understood to have probiotic effect (Fuller, 1989). Fermented foods are more nutritious than unfermented ones (Joshi et al., 1999; Sahlin, 1999) and foods prepared by using lactic acid bacteria (LAB) have better acceptability (Hang and Jackson, 1967). One of the reasons for the increasing interest in fermented foods now-a-days is their recognition to promote the functions of the human digestive system in a number of positive ways to maintain healthy life.

Probiotic is a functional food which is essential for good health. The term probiotic was introduced in 1965 by Stillwell and Lilly. Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001) and are also called friendly bacteria. Most probiotic microorganisms belong to the lactic acid bacteria (LAB), such as *Lactobacillus* spp., *Enterococcus* spp., or to the *Bifidobacterium* (Klein et al., 1998). Different bacterial species belonging to the *Lactobacillus* genus are part of the human and animal commensal intestinal flora (Zoetendal et al., 2006). Most of the probiotics are related to the *Lactobacillus* and *Bifidobacterium* (Reid et al., 2003; Guarner et al., 2005). However, to consider use of different strain as probiotics it should survive the upper digestive tract and must be capable of
surviving and growing in the intestine (acid and bile resistant), safe for human consumption, production of antimicrobial substances like bacteriocins to reduce pathogenic microorganisms and should be able to adhere to the epithelial cells (Morelli, 2000; Guarner et al., 2005). They should also be durable enough to withstand the stress of commercial manufacturing, processing and distribution.

Several health benefits associated with the consumption of live probiotic bacteria have been reported (Gomes and Malcata, 1999). These benefits include controlling intestinal infections, improving lactose utilization, and lowering blood ammonia levels (Ebringer et al., 1995). Probiotic bacteria can beneficially influence the immune system, and lower serum cholesterol levels (Ouwehand et al., 2002; Liong and Shah, 2005). Due to the wide range of therapeutic benefits there has been an increase in the incorporation of probiotic bacteria in a wide variety of food products including yogurt, cheese, drinks and dietary supplements (Ong et al., 2006).

At present the major focus of the researchers is on the identification of new strains of probiotic microorganisms and to use them in new product development to offer consumer a choice of probiotic products. Isolated strains can be identified either by phenotypic characterization or molecular identification by 16S rDNA gene sequencing. The 16S rDNA gene is nearly 1540 bases long and most reliable approach to identify bacteria on a phylogenetic basis and this approach was first proposed by Woese in 1987.

The Lactobacillus genus consists of a genetically and physiologically diverse group of rod-shaped, Gram-positive, non-spore forming, non-pigmented (Hasan and Frank, 2001), catalase negative and microaerophilic to strictly anaerobic (Vernoux et al., 2003) lactic acid bacteria (LAB) that have widespread use in fermented food production (Azcarate-Peril, 2001) and are considered as generally recognized as safe (GRAS) organisms and can be safely used for medical and veterinary applications (Fuller, 1989).

The lactobacilli isolated from dairy products have shown a long history of safe use (FAO/WHO, 2001). They are used widely as starter cultures in the food industry, e.g. fermented milk or meat products, alcoholic beverages, sourdough and silage (Carr et al., 2002). In raw milk and dairy products viz. cheese, yogurt and fermented milks, lactobacilli are naturally present or added to generate a health
benefit for the consumer and yogurt is one of the best-known foods that contain probiotics (Oskar et al., 2004).

Milk and milk products provide an excellent carrier for the probiotic microorganisms and most of them can readily utilize lactose as an energy source for their growth. Milk protein also provides important protection to the probiotic bacteria during passage through stomach (Charteris et al., 1998). In probiotics foods and supplements, the bacteria may have been present originally or added during preparation. Some examples of foods containing probiotics are yogurt, fermented and unfermented milk and some juices and soy beverages (Farnworth, 2005). There are also reports on use of probiotic ice cream, cheese, infant formulas, breakfast cereals, sausages, luncheon meats, chocolate and puddings and also in the form of capsules and tablets (Chan et al., 2000). Yogurt is a dairy product, produced by bacterial fermentation of the milk. The bacteria used to make yogurt are known as yogurt cultures i.e. *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture. Yogurt in human nutrition not only provides nutritive effect of milk but also shows its beneficial for intestinal microflora, as prophylactic and also for health (Marshall et al., 1984; Buttriss, 1997). Varieties of fruit yogurt have been formulated as probiotic fruit yogurts, and the survival of probiotic bacteria in yogurt have been investigated during cold storage (Kailasapathy et al., 2008). The functionality of yogurt increases with the addition of probiotic microorganism. Yogurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6, vitamin B12 and minerals (da Mota et al., 2000; Wall et al., 2006).

The term nutraceutical includes the substances which are traditionally recognized as nutrients and have positive physiological effect on the human body. These are the natural bioactive chemical compounds which have health promoting, disease preventing and other medicinal properties (Srividya et al., 2010). Fruits play an important role in the human diet and the presence of phytochemicals in fruits and their strong antioxidant potential in scavenging free radicals have generated tremendous attention among scientists (Rangkadilok et al., 2005). In India, pomegranate, apricot, persimmon and damson plum grow wild and in cultivated form in Himachal Pradesh, Jammu & Kashmir and Uttarkashi in Uttarakhand state. Himachal Pradesh is known as the fruit bowl of India and is one
of the major producers of fruits and vegetables. Among different fruits, wild apricot, wild raspberries, damson plum occupy an important place and are highly rich in antioxidants and nutrients. In Himachal Pradesh, fruits are generally grown in district Shimla, Mandi, Kullu, Chamba and Sirmour (Bhrot, 1998; Gupta et al., 2012). Owning to their short harvest time and highly perishable nature, apricots are mostly used for preparation of different value added products and preparation of fermented liquor (Gupta et al., 2012). Apart from retaining nutritional values these wild fruits are also rich in various medicinal properties (Beceanu, 2008).

The wild fruits are still underutilized in the state and as a result of this a lot of natural produce goes as waste. So the wild fruits viz. apricot (*Prunus armeniaca*), raspberries (*Rubus ellipticus*), damson plum (*Prunus domestica*) and jamun (*Syzygium cumini*) can be used for the development of fruit nutraceutical supplemented probiotic products. The primitive societies viz. gujjars, bakarewals, gaddis, ladakhis and kinnars residing in the Himalayan range of Jammu & Kashmir and Himachal Pradesh where temperature falls below −40°C in winter and the intensity of ultraviolet radiations are comparatively high have been using these fruits since ancient times (Kaur and Maini, 2001). These fruits have tremendous medicinal value and are used in curing various diseases. Consumption of fruits has significant health promoting effects and reduces the incidence of cardiovascular diseases, cancer and various degenerative diseases and are most cherished bounties of the nature (Bhattacharya et al., 1997; Ilavarasan et al., 2001; Manonmani et al., 2002; Li et al., 2008). The colour, texture, aroma and sweetness make them important and they are also the good source of dietary fiber, minerals and vitamins.

A number of studies have reported increase in production of free radical increases in human exposed to the environment with high altitude. The free radicals may be very damaging since it induces oxidation, which cause membrane damage, protein modification and DNA damage (Siems et al., 1995; Wang et al., 1996; Halliwell and Gutteridge, 1997; Marnett, 1999; Stadman, 2004; Valko et al., 2006). The oxidative damage is considered to play a causative role in aging and several degenerative diseases e.g. heart disease, cataracts, cognitive dysfunction and cancer (Toshniwal and Zarling, 1992; Lyras et al., 1997; Jenner, 2003; Sayre et al., 2001). The defense system that prevents the body from damage by the free radicals is called antioxidants (Kunwar and Priyadarsini, 2011). Antioxidants are
nutrients in food that protect our cells from damage from free radicals and prevent oxidation of the oxidizable substrate. Antioxidants are very effective as they can donate their own electrons to ROS (reactive oxygen species) and thereby neutralizing the adverse effects of the latter (Kohen and Nyska, 2002). The antioxidant protection system involves a variety of components, both endogenous and exogenous, that function interactively and synergistically to neutralize free radicals (Jacob, 1995). Endogenous antioxidants are bilirubin, thiols, ubiquinone, uric acid, NADPH, NADH and enzymes and exogenous include vitamin C, E, β-carotene and polyphenols (Percival, 1998).

The probiotic products face the problem of variation in viability or activity of cultures in various developed products. Therefore, it is essential to study the use of mixtures of microorganisms and their differential survival, to ensure a long shelf-life and controlled release of probiotic bacteria to their site of action in the gastrointestinal tract. During gastrointestinal tract passage, the cultures are required to tolerate low pH of stomach and antimicrobial activity of the bile salts. It is important to find methods for enhancing the viability of microbial cells in the digestive tract and one of them is to use microencapsulated probiotic cultures (Shahidi and Han, 1993). Microencapsulation is a technique of entrapping particles of solids, droplets of liquids or gaseous material with the help of protective shell or coating (Umer et al., 2011). The methods used for microencapsulation of microbial cultures include spray drying, emulsification and extrusion. In spray drying technique, a solution containing probiotic cells is dissolved in polymer matrix followed by drying to form spherical microparticles (Kailasapathy, 2009). In emulsification method an emulsifier, a surfactant and solidifying agent (calcium chloride) is added to the emulsion (De Vos et al., 2010). In extrusion method a solution containing probiotic cells are dropped through a nozzle at high pressure (Krasaekoopt et al., 2003). The use of high temperature in spray drying technique effect microorganism survival, whereas, emulsification and extrusion method gives a high survival rate of the microbial culture (Krasaekoopt et al., 2003; Chen et al., 2007). Out of above methods the extrusion method is simpler and cheaper (Krasaekoopt et al., 2003). Different materials are used to encapsulate probiotic cells viz. alginate, k-carrageenan, starch, gelatin, chitosan, milk proteins, gallan gum and xanthan gum (Burgain et al., 2011). Microencapsulation of probiotics with alginate or other gels improves the survival of microorganisms in probiotic
products and in human digestive system (Krasaekoopt et al., 2003, 2006; Crittenden et al., 2006). A probiotic bacteria encapsulated in 3 % sodium alginate survived in fruit juices (orange and apple) up to six weeks during storage, whereas, free probiotic bacteria lost their viability within five weeks (Ding and Shah, 2008).

The major focus of study is on identification of new strains of probiotic microorganisms from household dairy samples and to use them in new probiotic and antioxidant rich fruit supplemented probiotic product development. The probiotic products designed can provide the digestive aid for better absorption/digestion of the food, relief from constipation and reduction of allergic symptoms. The wild fruits selected in the present study are still underutilized in Himachal Pradesh, India. The antioxidants of fruit supplemented probiotic products can provide health promoting effects and reduces the incidence of aging and several degenerative diseases e.g. heart disease, cataracts, cognitive dysfunction and cancer by neutralizing free radicals in long run.

Keeping in view the importance of above work, the present study is endeavored to produce milk based probiotic and fruit nutraceutical supplemented products and investigate their storage stability with the following major objectives:

1.1. Objectives

I. Isolation of Lactobacillus from dairy samples and its characterization for probiotic properties.

II. Development of milk based probiotic products using free and microencapsulated culture and their storage stability study.

III. Development of milk based probiotic products with nutraceutical supplements of selected fruits and their storage stability study.