Review

Literature
2.1 Historical and Taxonomic development

Tsiklinsksy (1899) is accredited with the isolation of thermophilic actinomycetes from decaying straw. One of the species isolated from decaying straw sample produced chains of spores, which is designated now as *Streptomyces*. The other culture recognized as *Thermoactinomyces vulgaris*, formed round spores borne terminally or laterally on hypha and was believed to be widely distributed in nature. The organism was later classified as *Micromonospora vulgaris* (Waksman et al., 1939). The *M.vulgaris* grew at 48-60°C, the optimum temperature for the growth being 57°C whereas no growth occurred at 70°C. Henssen (1957) developed several methods for isolation and cultivation of thermophilic actinomycetes and discovered several new genera and species of *Thermoactinomycetes*.

According to the classification based on chemotaxonomy and numerical taxonomy, actinomycetes are placed under order actinomycetales, which is divided into eight aggregate groups (Goodfellow and Cross, 1984). Four of the groups are: (I) Streptomycetes, (II) Maduramycetes, (III) Micropolysporas and (IV) Thermomonosporas, which includes thermophilic actinomycetes. Actinomycetes have also been classified on the basis of spores produced on mycelium, and therefore, two groups, namely monosporic thermophiles and polysporic thermophiles have been recognized (McCarthy, 1985). The monosporic thermophiles included *Saccharomonospora*, *Thermomonospora* and *Thermoactinomyces* whereas *Micropolyspora*, *Streptomyces* and *Actinomadura* formed the polysporic thermophilic group.
The genus *Thermoactinomyces* first described by Tsiklinsky (1899), is spore producing and can survive a temperature of 100°C for 20 min. Several species of *Thermoactinomyces* are thermophilic aerobes and characteristically possess single spore on both substrate and aerial hyphae. The presence of single spore led the type species to be erroneously classified with the mesophilic Micromonospora for many years (Carboz *et al.*, 1963). The controversy was not resolved until it was shown that the spores of *Thermoactinomyces* are typical endospores and quite different from the spores produced by other actinomycetes (Cross, 1968; Dorokhova *et al.*, 1970). The spores contain dipicolinic acid (DPA) and are structurally similar to endospores of bacteria and are heat resistant up to 90°C for 30 min. (Lacey and Vince, 1971). The resistance to higher temperature differentiates *Thermoactinomyces* from other spore producing actinomycetes. The lower G+C content and 16S rRNA oligonucleotide sequence of *Thermoactinomyces* resemble to those of *Bacillus*, and, therefore, both are closely related. *T. vulgaris* isolated by Tsiklinsky (1899) is a gram positive organism whose cell wall contains mesodiaminopimelic acid, lacks sugars like arabinose, madurose and xylose (Lechevalier *et al.*, 1971).

*Thermoactinomyces vulgaris* and *T. thalpophilus* were considered as synonyms by Kuster and Locci (1963). *T. vulgaris* produced no endospores and were sessile. *T. vulgaris* did not utilize starch (Kou and Hertmam, 1967). The amylase producing and non-producing isolates were placed into two species which differed in their ability to degrade tyrosine, arbutin, chitin and esculin (Kurup *et al.*, 1975). The amylase producer was named as *T. vulgaris* whereas non-amylase producer as *T. candidus* which also produced spores on short sporophores. Consequently, it was
agreed that Kurup's concept of *T.vulgaris* corresponds to *T.thalpophilus* (Lacey and Cross, 1989) and *T.candidus* is identical to Tsikinsky's concept of *T.vulgaris*.

**Thermomonospora**

The generic name *Thermomonospora* was proposed by Henssen (1957) for certain thermophilic actinomycetes species which she found in rotten cow and sheep dung. The isolated strains differed from mesophilic monosporic genus *Micromonospora*, which characteristically lacks aerial mycelium. Henssen suggested the specific name, *fusca* to a common thermophilic actinomycete which she isolated from high temperature compost and placed in the genus *Thermomonospora*, because of their ability to form spores on the aerial mycelium. *T.fusca* was later on isolated in pure culture and was described in detail by Crawford and Gonda (1977).

A comprehensive numerical taxonomic survey of *Thermomonospora* and related organisms were done by McCarthy and Cross (1984). *T.curvata* was included in this genus while *T.alba* of the same genus was termed as "white Thermomonospora group" because of their white aerial mycelium. A reddish brown pigment producing colonies recognized as *T.chromogena* was included in this genus because of the similarity in the cell wall composition and morphology. The chemical analysis of *T.chromogena* and *T.mesophila*, however, supported their transfer to a revised genus, *Microtetrastroma* (Kroppensted et al., 1990). The morphology and natural habitat of *Thermomonospora* resemble to *Thermoactinomyces* to a greater extent, but the presence of endospores in *Thermoactinomyces* separates it from *Thermomonospora* and a few commonly found monosporic
actinomycetes. Thermophilic *Thermomonospora* are found in overheated substrates such as bagasse, compost, fodder and manures. They are, however, abundant in mushroom compost and are highly cellulolytic (McCarthy and Cross 1984; McCarthy, 1987). Recently, Zhang et al. (1998) using phylogenetic, chemotaxonomic and phenotypic studies have reclassified the *Thermomonospora* and *Microtetraspora*, where they proposed the transfer of the genus *Thermomonospora fusca* and *T.alba* to a new genus *Thermobifida* gen. nov. which belongs to the family, Nocardiopsaceae, as *Thermobifida fusca* comb.nov. and *Thermobifida alba* comb.nov.

**Saccharomonospora**

The species *Saccharomonospora viridis* was classified in the genus *Thermomonospora* by Kuster and Locci (1963). This genus possesses type III cell wall except for the species *Thermomonospora viridis* which exhibits type IV cell wall. It produces predominantly single spore on aerial hyphae. Spores are heat sensitive, non-motile, either sessile or formed at the tip of simple unbranched sporophores. On the agar media, a branched vegetative mycelium forms leathery colony, usually covered with aerial mycelium and spores remain densely packed with the hyphae. The aerial mycelium is initially white which later on becomes grey green to dark green or bluish green. At the later stages of growth, pigmentation diffuses into the surrounding medium. They do not show activity against cellulose where as casein, gelatin, starch, xylan and tyrosine are degraded.

**Saccharopolyspora**

The genus was first described by Lechevalier et al. (1961) and produces short chain of spores both on substrate and aerial
mycelia. Cell wall of *Micropolyspora* contains, alanine, glutamic acid, glucosamine, arabinose and galactose. The presence of mycolic acids in *M. brevicatena* and *M. fascifera* and the absence of mycolic acid in *M. faeni* and *M. rectivirgula* indicates heterogeneity among the species. The characteristics of *M. faeni* and *M. rectivirgula* have been fully described and clearly conform to the definition of genus *Micropolyspora* (Cross and Goodfellow, 1973). The difference however, between species was confirmed by Collin *et al.* (1988) who found menaquinones compound in *M. faeni* as found in *Streptomyces* while *M. brevicatena* had a mixture of tetrahydromenaquinones with six and eight isopyrene units like in *Nocardia*.

The epithet, *rectivirgula* was first published by Krasilnikov and Agre (1964) for the species of *Thermopolyspora*. Prauser and Momirova (1970) proposed the transfer of *Thermopolyspora* to *Micropolyspora*. Another epithet proposed was *faeni* as species of *Thermopolyspora* by Cross (1968) for the isolates that were obtained from hay samples. *Micropolyspora rectivirgula* and *M. faeni* had no differences in their morphology, physiology, immunological characteristics and susceptibility to phages (Dorakhova *et al.*, 1970). Detailed taxonomic studies by Kurup (1981) confirmed that these two species represent a single taxon.

According to the International Code of Nomenclature of Bacteria (Lopage *et al.*, 1975), when two taxa of the same rank are united, the oldest legitimate name should be retained. The type species of *Micropolyspora* is now therefore, known as *Nocardia brevicatena*. Kurup and Agre (1983) proposed a new genus *Feania* to replace *Micropolyspora rectivirgula* by *Faenia rectivirgula* which was later on accepted. *Saccharopolyspora rectivirgula* was reclassified as *Faeni rectivirgula* by Korn-Wendish *et al.* (1989).
Faeni rectivirgula is commonly found in fodder, grains, sugarcane bagasse and soil.

2.2 Thermophilic actinomycetes in Environment

Thermophilic actinomycetes undergo an interesting cycle in nature with regard to their dispersal and growth. They grow at sites of high temperature, such as, in compost, manure heaps and self-heating hay or grain. The vegetative phase ends with the formation of large number of spores, which returns to the field and pastures and contaminates soil directly or through soil dust, plant materials and hay. While passing through the intestinal tract of animals, spores survive and remain inactive but viable.

2.2.1 Manure and Compost

Natural manures which do not undergo self-heating process can not be excepted to harbour rich flora of mesophilic organisms. However, if such a process takes place, thermophilic actinomycetes become predominant. Activities of the mesophilic species raise the temperature above 40°C and develops humid conditions favourable for the growth of thermophilic actinomycetes and fungi (Lacey, 1973). The mushroom composts are usually made of straw-rich horse manure, wheat straw and chicken manure. After pasteurization, the mushroom compost is inoculated with Agaricus bisporus and is allowed to incubate at 45-50°C for 4 to 8 days during which thermophilic actinomycetes and moulds develop in large quantities. The isolation of thermophilic actinomycetes from such substrates by spreading dilutions on plates of non-selective nutrient medium is a tedious job, especially when the number of thermophilic actinomycetes are low. Thermomonospora chromogena was isolated by McCarthy and Cross (1981) from mushroom compost by spreading diluted
suspension of compost on agar media containing selective agent, kanamycin (25 μg/ml). The selective kanamycin not only suppresses the growth of other thermophilic actinomycetes but also reduces the growth of associated bacteria. The air released from the fermentation tunnel during spawning of mushroom compost collected by Burkard spore strap slide sampler by Bogart et al. (1993) showed 4.6 ± 3.2x10^5 spores per liter of air of actinomycetes while the fungal spores count was low. The isolation of *Thermomonospora curvata*, *T. fusca* and *T. alba* from the mushroom tunnel indicated their involvement in the etiology of Mushroom worker’s lung (MWL). The spores of *Agaricus* used for spawning is also considered as a causative agent in Mushroom worker's lung (Matsui et al., 1992). Thermophilic actinomycetes from manure heaps have been the subject of extensive studies since Henssen (1957) reported the presence of *Thermomonospora* in rotten cow and sheep dung.

The cellulolytic actinomycetes present in soil or manure samples were isolated by enriching the medium with finely cut pieces of whatman No. 1 filter paper in 10 ml of sterilized water in tightly plugged flasks followed by incubation at 55°C. The species were later identified by Hussein et al., (1996). The cellulolytic thermophilic strains of actinomycetes were also isolated from waste and mushroom composts in Germany (Korn-Wendisch et al., 1995). They had type III cell wall and phospholipid was of type PII. Mycolic acid was however, not present. The chemotaxonomic markers indicated that these organisms represented a new genera of the order actinomycetales, for which the name *Thermocrispum* was proposed. Actinomycetes isolated from horse manure included *Thermomonospora fusca*, *T. alba* and *Micromonospora* spp. The isolated species of thermophilic actinomycetes hydrolyzed xylan,
gelatin and Tween-80. The organisms grew in straw and increased temperature from 27°C to 63°C during biodegradation activity. The rise in temperature helps in eradicating the phytopathogenic organisms in the compost plant residues (Kukolya and Hornok, 1997). More than hundred cultures of TAs belonging to six species namely *F. rectivirgula*, *S. viridis*, *T. thalpophilus*, *T. vulgaris*, *S. thermoviolaceus* and *T. curvata* have been isolated from 76 different samples of municipal solid waste and were analysed for their colony characters as well as the biochemical reactions (Agarwal *et al.*, 1997).

### 2.2.2 Actinomycetes in soil

Actinomycetes are very common in soil and actively degrade complex polymers such as cellulose and chitin (Okolo *et al.*, 1996; Hussein *et al.*, 1996; Fett *et al.*, 2000). The isolation and evaluation of population density in the soil has conveniently been performed by dilution plate method. The actinomycetes in soil ranged between 10-50% of the total microbial colonies including thermophilic actinomycetes. Humus and pH (6.5-7.0) showed positive influence on the growth of actinomycetes. *Saccharomonospora* was most frequent in relatively humus-poor alkaline soil (pH 7-7.5) (Kawa *et al.*, 1988). However, Li-Hua *et al.* (1996) observed upto 90% actinomycetal diversity in soils of Yunnan, China. The distribution of actinomycetes in soil is also affected by pedological factors like depth, type of soil and the quantity and quality of organic matter (Sabaou *et al.*, 1992). The growth and germination of endospores of actinomycetes is also greatly affected by the moisture content of the soil along with organic carbon, nitrogen (N), pH and temperature of the soil (Babich *et al.*, 1996). Sand silt loam soil and silt clay loam soil inoculated with *T. thalpophilus* showed more recovery in silt clay
loam soil with a moisture content ranging from 5-50% (w/w) at 30 to 40°C. Addition of sterilized farm yard manure in sand silt loam further improved the growth of actinomycetes with a significant increase in organic carbon and N content of soil (Jackson and Ball, 1998). Organic matter rich soil in Qatar yielded *Thermoactinomyces* sp. including *T. vulgaris, T.thalophilus, T.putidus* and *T.sacchari*. The optimum temperature for germination was found to be 55°C with pH 7.5-8.5 (Shoreit, 1992). In Egypt, Ammer *et al.* (1988), selected the desert of Aswan to see the effect of seasonal variation on the population of thermophilic actinomycetes in soil. The highest actinomycetes count were recorded during summer followed by spring. The humid condition of spring probably favoured the growth of thermophilic actinomycetes and in Autumn and winter, actinomycetes counts was low probably due to dry and low temperature. *T.thalophilus* and *S.viridis* were isolated from soils of Vietnam and Japan and were found to exhibit amylase activity (Mai *et al.*, 1992; Takashashi *et al.*, 1992). These naturally occurring actinomycetes in the soil showed fungistatic property which was negatively influenced by indiscriminate use of pesticides in agricultural practices. The negative effect of pesticides can be removed by amending the soil with organic matter (Pedziwilk, 1995).

### 2.2.3 Actinomycetes in water

The spores of actinomycetes enters rivers and lakes through soil and settles in marine sediments. The endospores of *Thermoactinomyces* exhibit extreme longevity and remain viable for hundred of years in dry soil and sediments of cold anaerobic lakes (Unsworth *et al.*, 1977). River water when stored in reservoir showed a rapid decline in the number of *Streptomycetes* but the number of *Micromonospora* remain fairly constant for longer
period of time in fresh water (Burman, 1973). The settled spores in deep mud and sediment samples at the depth of 4,400 m were studied by Attwell (1973), where the spores of *Thermoactinomyces* species were predominant in all the samples. *Micromonospora* and *Streptomyces* dominated the lake sediment of middle plateau of Yunnan (Jiang and Li-Hua, 1996). It has been established that lake sediment harbour oldest endospores belonging to *Thermoactinomyces* and *Clostridia* spp. The recovery of *Thermoactinomyces* species from deep sea-sediments from Japan indicated the movement of terrestrial material into deep ocean (Colquhoun *et al.*, 1998). Recently, their presence in a covered and heated swimming pool was observed by Moreno *et al.* (1997) who confirmed their involvement in allergic response of patients who were the regular member of the swimming pool. The patients developed precipitin response against *Neurospora* spp. and *Micropolyspora faeni* isolated from the water of swimming pool.

### 2.2.4 Fodder, Hay and grain

Different agro-industrial environment dealing with moldy materials like hay, grain, cotton, cereal crops and bagasse harbours significant number of culturable microbial population. The waste of these substrates after processing of agricultural product possess the mixture of sugars, proteins, fats, cellulose, lignin, pectin and chitin. According to Mishra and Upadhyay (1998), plant materials alone, contain 28-49.3% of cellulose, 22-50% of hemicellulose and 13-35% of lignin which provides the best source of energy for the decomposing microorganisms. Therefore, in farm houses, hay and grains get contaminated by a wide range of decomposing actinomycetes through soil, compost and dust.
The process of self-heating and moisture content of hay or fodder favours the development of particular microorganisms. Moisture content of the hay samples (25-30%) results in temperature increase up to 45-50°C and therefore, favours the growth of some Streptomyces species. When temperature rises up to 60 to 70°C, Streptomyces are replaced by thermophilic actinomycetes of other genera (Festenstein, et al., 1965). During storage of moist wheat or corn grain, self heating takes place and several thermophilic actinomycetes develop, mostly on the surface of the silage because surfaces are usually exposed to the air and absorbs moisture (Dutkiewicz et al., 1989). T.vulgaris and F.rectivirgula were commonly isolated from the stored hay and grain samples in farm houses of Finland (Kotimma et al., 1987).

Stored fodder (hay and grain) in 34 dairy farms in France showed the presence of Streptomyces and Aspergillus spp. The colonies were fewer on Petriplates exposed to farm environment with bran drying system than those detected through traditional storage system. However, drying of fodder before storage helped in preventing the molding of material in large scale (Dalphin et al., 1991). The contaminated organic dust from hay, silage and grain contains a considerable population of thermophilic actinomycetes. Lacey (1973) isolated S.viridis and F.rectivirgula from straw samples, the population density being 14% and 42% respectively. In some other studies, F.rectivirgula have been found as a dominating species in dairy farms (Brummund et al., 1988), where hay probably was believed to be a preferential substrate for the growth of F.rectivirgula (Ranalli et al., 1999).

During an investigation of settled dust from rice and wheat straw processing units in Shanghai, China, the level of microbial contamination was found greater in hay dust than in rice dust.
samples. The species isolated were *Aspergillus*, *Cladosporium*, *Penicillium* and *Trichospora* (Shen et al., 1991). Rice husk samples collected from rice mills of Punjab, yielded *T. candidus* in 32% of the 112 samples followed by *S. viridis* (31.2%), *T. vulgaris* (8.7%) and *T. fusca* (4.4%) (Singh et al., 1991). *T. fusca* is known for solubilizing lignin present in rice or wheat straw to sustain its growth (Trigo and Ball, 1994). *T. vulgaris* was reported as a commonest species of thermophilic actinomycetes obtained from rice straw samples in north western India as demonstrated by Gangwar et al., (1989). They also found largest populations of *T. thalpophilus* in paddy straw and a high concentration of *F. rectivirgula* in hay samples. *T. sacchari* was common in sugarcane bagasse samples. The presence of *T. sacchari* in sugarcane mill environment was reported by Boiran et al. (1988) explaining its association with bagasse where bagasse provided most favourable substrate for the growth of *T. sacchari*. Bagasse have also been used as a sole carbon source by *Thermomonospora curvata* which posses an enhanced extracellular enzyme producing ability (Stutzenberger, 1994). Khan et al. (1995) noted *T. sacchari* and *S. viridis* as the major species present in bagasse handling workers.

The allergic species of thermophilic actinomycetes were isolated from vegetative substrate samples from Anambra and Enugu state of Nigeria. *T. vulgaris* and *T. thalpophilus* were found in rice straw, *Saccharopolyspora rectivirgula* (*F. rectivirgula*) in hay and *T. sacchari* in maize silage (Unaogu et al., 1994). The prevalence of clinically important thermophilic actinomycetes in agro-environment suggests that the workers exposed to these actinomycetes may develop farmer's lung disease.
2.3 Aerial prevalence of thermophilic actinomycetes

Organic substrates in agro-environment and industries usually get molded while they remain in field or during storage (Caroline *et al.*, 1995) The decomposing microorganisms on different vegetative substrates can easily be detached by disturbance of substrate or through air movement. The association of clinically important airborne thermophilic actinomycetes in agro-environment are considered to be responsible for respiratory disorder in workers. Farm houses storing hay and grains have dusty environment carrying high spore density of *T.vulgaris*, *F.rectivirgula* and *Aspergillus umbrosus*, which causes respiratory problems in farmers (Kotimaa *et al.*, 1987).

Saw dust and rice husk based litter in poultry farms are also the source of clinically important thermophilic actinomycetes. Therefore, airborne *T.vulgaris*, *S.viridis* and *F. rectivirgula* were isolated from the sites with decomposing rice husk litter near poultry sheds (Gangwar *et al.*, 1989). Dairy farm environment have also been found dominated by *S.viridis* whereas lower count of *T.vulgaris*, *T.thalpophilus* and *F. rectivirgula* were noted in area of fodder storage by Dalphin *et al.* (1991). They used five stage Anderson sampler for surveying 34 dairy farms in France. Genera like *Streptomyces* and *Aspergillus* were frequently encountered. Farms with bran drying system showed greater frequency of actinomycetes than in the traditional storage system. The workers exposed to the dried fodder system showed less acute symptoms like dry cough, asthmatic symptoms and irritation of eyes in comparison to the workers of traditional storage system indicating that fodder drying in farms might have protected them from farmer’s lung disease (Dalphin *et al.*, 1994).
Straw, grain and other feeding materials in stables possess highest load of respirable particles which contains thermophilic actinomycetes (Clarke and Madelin, 1987). Clinically important thermophilic actinomycetes in the air and hay samples were investigated from the environment of affected equines by Gangwar et al. (1989). During stabling period, equines get exposed to microorganisms growing on hay, which provide a favourable atmosphere for their growth. Highly respirable particles released by hay was found to contain spores of thermophilic actinomycetes and fungi (Clarke and Madelin, 1987). Therefore, exposure to the molding hay has been considered as the important cause of chronic obstructive pulmonary disease (COPD) in equines. These allergic respiratory diseases were also demonstrated by precipitating antibodies against *F. rectivirgula* in the sera of equines, where 78% equines showed respiratory problems (Khan et al., 1985). Subsequently precipitins against thermophilic actinomycetes in horses were also demonstrated by Madelin et al. (1991) from an English racing stable. In the susceptible horses, neutrophils invade the lung and accumulate in the lumen of airways, particularly bronchioles (Robinson et al., 1995).

The stable workers exposed to moulds are also at risk of developing hypersensitivity reactions. An 11-year-old girl, after a brief exposure to horse stable environment at riding school, Denmark, exhibited symptoms of farmer's lung disease (Kristansen and Lohez, 1991). These hypersensitivity reactions are of great concern for veterinary practitioners as well as physicians dealing with respiratory diseases. Therefore, to evaluate health hazards for workers in swine confinement building, Donham et al. (1986) used 37 mm cassette filter for trapping aerosols, which yielded mean total aerosol of 6.3 μg/m³ and mean respirable...
aerosol of 0.5 μg/m³ of air sucked, containing grain particles and faecal matter. The settled dust culture showed highest count of *Verticillium* spp. (5×10² cfu/mg). In a similar study, airborne microorganisms in pig farms were observed by Crook *et al.*, (1991) ranging from 10⁵ to 10⁷ cfu/m³ of air. The predominant organisms were bacteria with a few fungi and thermophilic actinomycetes due to which workers showed respiratory symptoms, chest tightness and wheezing with nasal and eye irritation. The cow houses in Finland showed airborne viable spores of thermotolerant fungi and thermophilic actinomycetes. High concentration of ammonia and carbon dioxide between 2.8-15 ppm and 2200-3200 ppm respectively were found in traditional cow houses. Where as, modern cubicle cow houses provided a better working environment with regard to airborne hazards (Lauhelainen *et al.*,1997).

Aerial prevalence of thermophilic actinomycetes in cotton mill was noted by Lacey and Lacey in 1987, where he isolated *T.vulgaris*, *F.rectivirgula* and *S.viridis* from cotton mill environment. Gangwar *et al.* (1989) found *T.vulgaris* as prevalent species in all the sections of a cotton mill near Delhi, India. Similarly agro-based industries like sugarcane mill harbour clinically important thermophilic actinomycetes, where bagasse provides the sole carbon source for the growth of thermophilic actinomycetes especially *T.sacchari* (Boiran *et al.*, 1988; Khan *et al.*, 1995). The domestic waste contain high concentration of airborne dust, bacteria and fungal spores where workers involved in manual sorting of unseperated domestic wastes experiences symptoms like asthma, eye irritation, cough etc. (Paulsen *et al.*, 1995). The air-spore concentration of thermophilic actinomycetes was high in crowded area of Cairo city than in the quieter
residential areas, where Diab and Khalid (1991) identified *S. viridis*, *T. vulgaris* and *F. rectivirgula*. These pathogenic species were also found in the air samples collected from a composting sites, located in the district of Graz, Austria (Haas *et al.*, 1999).

### 2.4 Antibiotic sensitivity

Thermophilic actinomycetes usually forms a significant component of the microbes in natural substrates which grow at a temperature above 50°C. The mouldy fodder, hay, grain and compost of natural plant materials are the substrates, from where the isolation of TAs is not an easy job. The spreading of serially diluted substrates on plate of non-selective nutrient media can be frustrated by the rapid growth of thermophilic bacteria and fungi which provide intense competition on the surface of isolation plates. However, when the number of TAs to be isolated are low, then it becomes necessary to use selective agents, which can be incorporated into isolation media to reduce the growth of certain undesirable microorganisms. Antibiotics, therefore, have been proved greatly effective in the isolation of thermophilic actinomycetes. Cycloheximide or polyenes have been used routinely to suppress fungal growth on plates containing actinomycetes and the addition of novobiocin has provided a highly selective medium for the isolation and enumeration of member of the genus *Thermoactinomyces* (Cross, 1968). Novobiocin (25 μg/ml) when added to the medium inhibits the growth of all the associated bacteria capable of growth at 50°C, whereas, cycloheximide or nystatin (50 μg/ml) in a suitable medium suppresses the growth of fungi. McCarthy and Cross (1981), isolated *Thermomonospora chromogena* from mushroom compost by spreading diluted suspensions on an agar medium containing kanamycin (25 μg/ml). The antibacterial drug
prevented the growth of other thermophilic actinomycetes on media incubated at 50°C and significantly reduced the growth of associated bacteria as well. Whereas sodium chloride and hippurate was used to isolate selectively *S. rectivirgula* by McCarthy (1985) and rifampicin was used to improve the recovery of *S. viridis* (Athalye *et al*., 1981). The clinically important thermophilic actinomycetes causing human actinomycetoma in Eastern India includes the species of *Nocardia* and *Streptomyces*. Most of these bacteria are therapeutically curable by sulfonamide, cotrimoxazole, erythromycin, streptomycin, ampicillin and tetracycline (Williams *et al*., 1989). The *in vitro* susceptibility testing of actinomycetes is however, often hampered by the slow and clumpy growth (in broths) of the organisms. However, Chaudhuri *et al.* (1997) studied both agar dilution and disc diffusion method and found both methods equally effective in detecting the sensitivity patterns of *Nocardia* isolates. The isolates were found sensitive to amikacin and ciprofloxacin. Therefore, the standardized susceptibility of actinomycetes to different antibiotic is necessary to ensure the selective isolation of particular species of actinomycetes which will help further to formulate guidelines for therapy of disease caused by actinomycetes.

2.5 **Clinical manifestation of Hypersensitivity pneumonitis**

Clinical description of farmer's lung disease was first recognized by Campbell (1932) as a peculiar respiratory illness in English farmers, which he attributed to white moulds present on hay and grain. Fuller (1953) recognized three different phases of farmer's lung disease. Phase 1, included cases with mild attack lasting only 1 to 3 days; phase 2 was characterized by recurring
episodes with increasing dyspnoea, cough and characteristic radiograph. Recovery from phase 1 and 2 could be achieved if exposure is avoided, while phase 3 was characterized by irreversible lung changes caused by exposure over a period of many years. Subsequently, Frank (1958) reviewed 127 cases of farmer’s lung disease and suggested that it is a hypersensitive condition which develops as a result of repeated inhalation of the organic dust. The first steroid therapy in a patient of farmer’s lung disease used by Cooper (1961) resulted in complete remission. This also provided as indirect evidence that lung disease is an immunological inflammatory disease.

Realizing the growing importance of the occupational nature of the farmer’s lung disease, the British ministry of agriculture issued a warning advising farmers to wear an efficient face mask while handling moldy hay or corn. The importance of such a preventive measure was also highlighted in an editorial in British Medical Journal (1961). A significant advancement in the laboratory diagnosis of farmer’s lung disease occurred when Pepys et al. (1965) used agar gel diffusion test for detection of antibodies in patient’s sera against antigen of moldy hay. The inhalation challenge with culture filtrate extracts of hay and fungi in patients with farmer’s lung were characterized by delay type of hypersensitivity reactions (Williams, 1963). He concluded that inhalation test with moldy hay extract were of value in diagnosis of farmer’s lung. Lacey and Lacey (1964) estimated that a person would inhale about three quarter of a million spores per minute while handling moldy hay in farm buildings. This led to the assumption that it is the massive exposure to spores which result in the development of farmer’s lung disease.
2.6 Incidence of Hypersensitivity pneumonitis

The occurrence of farmer’s lung disease and other forms of hypersensitivity pneumonitis is more common (Kryda and Emanuel, 1986). Most of the studies on farmer’s lung have been carried out in the Great Britain and Scandinavian countries and a few has been reported from U.S.A. and other parts of the world. Stains and Forhman (1961) reported an incidence of 193 cases per 100,000 farming population in Wales, 0.073% cases in south west England and 0.072% in east England. It was speculated that about 1000 new cases of farmer’s lung occur in England every year. The highest incidence of the disease occurred in males due to occupational exposure. Though it has also been reported in women (Homma et al., 1986; Depierre et al., 1988). A 62 year old farmer woman from northern east, a very rainy part of Turkey, was reported with respiratory distress for over 20 years. This allergic alveolitis was caused by moldy hazelnut leaves (Erakan et al., 1992). A brief exposure to the air borne molds in a riding school led to the development of farmer’s lung disease in a small girl in Denmark (Kristianssen and Lohaz, 1991). However, the population of young students in farming college in the state Tyrol, Austria, showed precipitin reactions against F. rectivirgula with a history of asthma in some of them (Prior et al., 1996) indicating that even young age groups showed a clear relationship between allergic sensitization and allergen exposure.

In a comprehensive study in Scotland, Grant et al. (1972) recorded the prevalence of farmer’s lung symptoms as 8.7% in west and 2.3% in east of the country. Terho et al. (1980) reported 50 cases of farmer’s lung disease per 100,000 in Finnish farmers. There were however, differences in the incidence based on rainfall and subsequent drying of hay. In a study from north Italy, 2.7% of
2932 agricultural workers reported symptoms suggestive of farmer’s lung disease (Saia et al., 1984). The investigation of 512 Swedish farmers from an area where grain was prominent crop, revealed that 19% population had febrile reactions from grain dust exposure and yearly incidence of allergic alveolitis was 2-3/10,000 of farmers (Malmberg et al., 1985, 1988). It was inferred that many symptomatic farmers had a syndrome due to grain dust rather than hay in farmer’s lung disease.

In the U.S.A., the incidence pattern of the disease appears similar to that found in England. There is much greater likelihood of farmer’s lung in winter following a hot summer with heavy rain when farmers feed the moldy forage to livestock (Emanuel and Kryda, 1983). Several reports from U.S.A. have indicated the prevalence of antibodies to antigens associated with farmer’s lung disease (Marx et al., 1978). The precipitin reactions to antigens of thermophilic actinomycetes among 124 dairy farmers in Vermont, USA were precipitin positive to M. feana (5.4%) and T. vulgaris (1.2%) in the suspected farmer’s lung patients (Cormier et al., 1985, 1986). An investigation by Marcer et al., (1983) from Northern Italy, observed that 36 farmers showed clinical history of farmer’s lung and 39 reported attacks of breathlessness which was associated with fever, after exposure to moldy hay. For the diagnosis of farmers lung, skin tests with hay extract were used by Edward et al., (1981). The clinical findings by Gangwar et al. (1991) suspected the farmer’s lung in dairy herd workers. Subsequently the precipitating antibodies against T. sacchari was found in workers exposed to sugarcane mill environment which suggested the prevalence of this organism in the agro-environment (Khan et al., 1995).
The FLD surveys in farming populations revealed that the prevalence of FLD ranged between 25 and 85 per 1000 individuals in Europe and between 4 to 30 per 1000 subjects in U.S.A. (Fink, 1987). The difference in prevalence rates of FLD reflect the difference in farming practices including crop cultivation, storage facilities, molding of hay and clinical awareness. Recently, Von Ehrenstein et al. (2000) reported that exposure to livestock and other environmental factors are the risk of the hay fever and asthma among children of farmers.

2.7 Etiology of hypersensitivity pneumonitis

Thermophilic actinomycetes incriminated in the etiology of farmer’s lung disease (FLD) includes, *F. rectivirgula, T. thalpophilus, T. vulgaris and S. viridis* (Pepys, 1969). *T. thalpophilus* and *T. sacchari* have been found as the causal organism of bagassosis (Lacey, 1971). Mushroom worker’s lung (MWL) were found to be associated with the spores of fungi and thermophilic actinomycetes prevalent in the mushroom composts. The thermophilic actinomycetes in mushroom composts included, *Thermomonospora fusca, T. alba, T. curvata* and *Thermocrispum* spp. (Bogart et al., 1993; Korn-Wendisch et al., 1995). Different forms of hypersensitivity pneumonitis are usually named according to the environment in which they occur or source of antigen where they are found.

Temporal relationship between symptoms and certain activities such as working with hay, bagasse and particular hobby or occupation exists which may produce a clue to the disease (Terho et al., 1987; Depierre et al., 1988). The remission of symptoms during the extended removal from the source of antigen supports the diagnosis. The various types and source of
thermophilic actinomycetes associated with hypersensitivity pneumonitis (HP) is listed in Table I. The major forms of HP are briefly described in the present review.

**Table-1 Thermophilic actinomycetes causing hypersensitivity pneumonitis**

<table>
<thead>
<tr>
<th>Thermophilic Actinomycetes</th>
<th>Source</th>
<th>Disease Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faenia rectivirgula</td>
<td>Moldy hay</td>
<td>Farmer's lung</td>
</tr>
<tr>
<td>Thermoactinomyces vulgaris</td>
<td>Moldy corn</td>
<td></td>
</tr>
<tr>
<td>T. thalpophilus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomonospora viridis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. thalpophilus</td>
<td>Moldy bagasse</td>
<td>Bagassosis</td>
</tr>
<tr>
<td>T. sacchari</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>Moldy vegetable</td>
<td>Mushroom</td>
</tr>
<tr>
<td>T. thalpophilus</td>
<td>Compost</td>
<td>Worker's Lung</td>
</tr>
<tr>
<td>S. viridis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. rectivirgula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. thalpophilus</td>
<td>Air-conditioning and humidification systems.</td>
<td>Ventilation System induced pneumonitis</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. viridis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.7.1 Farmer’s lung disease (FLD)

FLD is the most common forms of HP caused by inhalation of moldy hay contaminated with thermophilic actinomycetes and fungal spores (Pepys, 1969). Moist hay stacking or moist grain storage undergoes fermentation and therefore lead to the elevation
of the temperature. The raised temperature thus, selectively promotes the growth of thermophilic actinomycetes and fungi (Kotimma et al., 1987). Repeated inhalation of spores of these actinomycetes may lead to the development of FLD in susceptible individuals. The organisms responsible for the farmer's lung disease are, *F. rectivirgula*, *T. thalpophilus*, *T. vulgaris* and *S. viridis* (Gangwar et al., 1991; Gaur et al., 1992). A new thermophilic species named *Streptomyces hygroscopicus* isolated from haystack, moldy hay is also one of the causative agent of farmer's lung disease and is prevalent in Hubei and Shanghai, China (Lu et al., 1991). Cattle and equines also develop a condition similar to farmer's lung disease during winter when they are confined to stable and exposed to moldy hay (Khan et al., 1985; Robinson et al., 1995).

### 2.7.2 Bagassosis

Bagassosis results following prolonged inhalation of dried sugarcane fiber dust. Sugarcane waste after extracting the juice when allowed to stand for a long time is known to promote the growth of thermophilic actinomycetes (Lacey, 1971). Salvaggio et al. (1969) employed extracts of fresh and moldy bagasse samples for the detection of precipitin reactions in the sera of bagasse affected workers. More precipitins were recorded in bagasse affected workers than unaffected ones. They also demonstrated more precipitins against *T. vulgaris* in bagasse affected subjects. *Thermoactinomyces sacchari* was believed to be the main thermophilic actinomycetes present in bagasse and caused the acute respiratory illness (Lacey, 1971). This disease is similar to farmer's lung disease and antibodies against *T. sacchari* has been reported in sera of patients (Salvaggio et al., 1969). Recently, precipitins against *T. sacchari* and *F. rectivirgula* were
demonstrated in the sera of sugar mill workers in India (Khan et al., 1995). These organisms are suggested to be involved in sensitization of the bagasse workers leading to bagassosis.

2.7.3 Mushroom Worker’s Lung (MWL)

In United Kingdom, the term mushroom worker’s lung was introduced for the first time by Sakula (1967), where he described the clinical similarities of MWL with farmer’s lung disease. Mushroom compost is known to harbour a variety of thermophilic actinomycetes namely, *F. rectivirgula*, *T. thalpophilus* and *T. vulgaris* (Pepys, 1973). Spawning of mushrooms and mechanical agitation of compost (open trays) leads to high concentration of airborne spores of these thermophilic organisms which lead to MWL and has been observed in U.K. and France (Jackson and Welch, 1970; Berruchon and Owry, 1981). Kleyn et al. (1981) tried to correlate the isolation from mushroom compost and the etiology of mushroom worker’s lung. Since, their investigations did not show *F. rectivirgula* in the mushroom compost, it was questioned if this species is really an important etiological agent of MWL as widely believed. Philips et al. (1987) reported four cases of acute respiratory illness among workers of a factory in U.K where compost was being produced for mushroom cultivation. Specific IgG antibodies reactive to mushroom spores were recorded in the serum of a 38 year old woman, involved in mushroom cultivation. The spores of *Agaricus bisporus* were therefore suspected as the causative agent of MWL (Bogart et al., 1993; Korn-Windisch et al. 1995).

2.7.4 Ventilation system induced pneumonitis

Hypersensitivity pneumonitis among workers of contaminated airconditioning and humidifier system was reported
by Banaszak et al. (1970). The disease caused by exposure to these systems are generally called as humidifier's lung (Sweet et al., 1971; Fink et al., 1976). The water of the humidifier remains warm and therefore, become grossly contaminated with a variety of microorganisms. In most cases, thermophilic actinomycetes and fungi were isolated from humidifier water as reported by Arow et al. (1978). Thermoactinomyces, Aspergillus, Cladosporium and Trichoderma have been isolated from air cooling system. Faeni rectivirgula was isolated from contaminated ventilation system of an office (Banaszak et al., 1970). Subsequently, Fink et al., (1971) described two additional cases of the disease demonstrating precipitins against F. rectivirgula and T. vulgaris. HP resulting from contaminated air conditioners and cooling humidification system of factories were reported by number of workers (Kumar et al., 1981; Friend et al., 1977 and Ganier et al., 1980). The workers developed extrinsic allergic alveolitis in a printing press due to contaminated cold water of humidifier. The precipitins to the humidifier antigens were strongly positive in the suspected workers as shown by Robertson et al. (1987) and the lavage fluid from lung showed more than 70% lymphocyte in each case. Baur et al. (1988) used the tube immunoradiometric assay for detecting IgG in the sera of suspected patients against the extract of water humidifier system. They also isolated Alternaria tenius, Aureobasidium pullulans, Penicillium noctatum and Aspergillus from water.

The use of home ultrasonic humidifier has become widespread (Shiue et al., 1990; Volpe et al., 1991). The precipitin in the sera of five patients of HP against Candida albicans and Cephalosporium acremonium suggests that the home air condition is the main cause of humidifier fever (Suda et al., 1995).
2.8 Pathological findings

In chronic stages of farmer's lung patients, pulmonary biopsies show damage in alveolar epithelium and results in fibrosis of the lung. In general, the granulomas are found, which composed of macrophages derived cells and are characterized by a clustering of the epitheloid cells (Reyes et al., 1982). Induction of alveolitis requires a coordinated interaction of alveolar macrophages and lymphocytes. However, there are numerous reports that alveolar macrophages exhibit low accessory function and are poor antigen presenting cells or even suppress T-cells activation (Fireman et al., 1993; Yarbrough et al., 1994). In contrast, a granulomatous interstitial lung disease is of unknown origin in sarcodiosis where alveolar macrophages exhibit an increased ability to act as antigen presenting or accessory cells (Ina et al., 1990). Therefore, one can hypothesize that increased accessory function of alveolar macrophages is an immunobiological pre-requisite for granuloma formation (Gernot et al., 1997). Chest X-ray of a 35 year old farmer with complaints of productive cough and sputum showed reticulonodular shadows. He was found precipitin negative for M.faeni and biopsy specimen of right scalene lymph node showed epitheloid cell granulomas and spot-like calcification.

2.9 Bronchoalveolar lavage (BAL)

A number of interesting observations were made from investigation on BAL of hypersensitivity patients (Keller et al., 1984; Cormier et al., 1985; 1986). The activated T-cells were observed in BAL fluid of patients with active disease, but not in exposed asymptomatic individuals. The activated T-cells are likely to be the consequence of on going local immune response but not a marker of disease activity on progression. The higher number of
total cells found in BAL fluid of farmer's lung patients suggests an earlier manifestation of interstitial alveolitis (Koegh and Crystal, 1982). Non-smokers of dairy farms in Stockholm were analyzed by Larsson et al. (1988) whose BAL had an elevated proportion of lymphocytes, fibronectin, angiotensin converting enzyme and albumin. Increased angiotensin converting enzyme in the concentrated BAL fluid probably originates in alveolar macrophages as reflected by their activation (Eklund et al., 1986). The high concentrations of fibronectin was noted by Larsson et al. (1988) in a 440 Kd glycoprotein of plasma while extracellular matrix was found in alveolar macrophages (Rennard et al., 1981).

Fibronectin has chemotactic and stimulatory effect on fibroblast and thus affects the development of fibrosis in patients with interstitial lung diseases. The elevated albumin concentration in BAL fluid, however, indicates an increased alveolar capillary permeability (Eklund et al., 1986). Lymphocytosis and increased albumin concentrations have been reported in BAL fluid from asymptomatic farmers with no history of farmer's lung disease (Cormier et al., 1985, 1986). However, BAL test for the three subjects of extrinsic allergic alveolitis (EAA) showed more than 70% of lymphocytes and were precipitin positive to the humidifier and antigens to which they were exposed in their printing press (Robertson et al., 1987). Hypersensitivity pneumonitis due to the inhalation of mushroom spores in an old women had symptoms of cough, nausea, malaise, leukocytosis and reduced lung volume. The alveolitis was demonstrated by transbronchial lung biopsy as well as increased lymphocytes in the bronchoalveolar cough, respiratory distress, intermittent fever and had developed the clinical sign of fibrosis of the lung with BAL showing predominance of CD-8 cells (Eklund et al., 1986).
In Japan, patients of hypersensitivity pneumonitis showed an increased lymphocytes, predominantly CD4+ in BAL. T-helper cell count (CD4) to suppressor T-cell count (CD8) ratio was also found significantly higher as compared to control (Suda et al., 1995). Schuyler and Edward (1996) demonstrated that CD4+la, T-cells are responsible for transfer of experimental hypersensitivity pneumonitis. Their model of adaptive transfer of experimental hypersensitivity pneumonitis was mice, sensitized to M. feani and their T-cells were injected into recipient mice. The recipient mice exhibited exaggerated pulmonary histological response when they were intratracheally challenged to M. faeni. The sensitive mice when exposed to S. rectivirgula for weeks, developed granulomatous inflammation with increased lung weight and lymphocytes in total bronchoalveolar cells as compared to resistant strains of mice. The sensitive strain had significantly greater antigen-induced IL-12 and IFN-γ gene expression. Therefore, Gunnar et al. (1998) suggested that host factors are also important for expression of hypersensitivity pneumonitis.

The histochemical examinations of the lung of patients suffering from hypersensitivity pneumonitis revealed the follicular area in Bronchus-associated lymphoid tissue (BALT). BALT, contains mainly B-cells while the parafollicular area is comprised of predominantly the T-cells. These cells proliferates actively after antigen stimulation (Suda et al., 1999) and therefore, BALT development is likely to play an important role in the mucosal immune response. Pulmonary phagocytic effect in guinea pigs against T. vulgaris antigen has been studied by Milanowski et al. (1998). The BAL showed influx of neutrophils, lymphocytes and red blood cells to the lung and therefore results
in the enhancement of phagocytes which caused inflammation of the tissues.

2.10 Serodiagnosis of Hypersensitivity Pnuemonitis

The preparation of cultural filtrate antigens, its purification and immunochemical characterizations have led to the identification of major antigenic component among TAs (Reiss, 1986). The critical factors for obtaining consistent and reproducible antigenic extract includes, strain selection and standardization of incubation time, temperature, aeration and inoculum size (Boiron, 1988). Various reports claiming increased sensitivity and specificity of antigens proved that none of these preparations are standard. The crude antigenic mixtures however, has continuously been used in routine diagnosis of HP. All the thermophilic actinomycetes causing clinical disease in susceptible individuals may have some of their antigen identical or closely related. Wenzel et al. (1974) found that sera from patients of farmer’s lung disease (FLD) contain precipitating antibodies reactive against antigens prepared from several species of thermophilic actinomycetes. The cross-reactivity of extracellular antigen of F. rectivirgula, T. vulgaris, T. thalpophilus and S. viridis were detected by double immunodiffusion (DID) and cross immuno-electrophoresis tests (Kurup et al. 1976). In their tests, all the antigens from different genera reacted strongly with their respective homologous antiserum raised in rabbits. A very little cross-reactivity was seen among the antigen of different genera. Flectcher and Rondle (1973) tried to obtain serologically active material from culture of M. faeni. The results of immunodiffusion and immuno-electrophoresis suggested that antigen preparation for diagnosis of farmer’s lung disease should contain concentrated culture supernatant and extract of mycelium. Their attempt to
obtain serologically active material from spores were, however, unsuccessful. The hypersensitivity in farmers arises from the exposure to mycelial antigens, which are rich in carbohydrates, particularly, arabinose, galactose and glucosamine and a very little amount of antigenic protein. The proteolytic activity of thermophilic actinomycetes, however, were demonstrated by the presence of antibodies against two proteolytic enzymes produced by *T. candidus* in the sera of FLD patients (Robert *et al.*, 1983).

The purified antigenic protein from *M. faeni* used in immunoblotting assay (Aznar *et al.*, 1988) were obtained from sera of FLD patients. The predominant immunoglobulin class was IgG with 20 bands in a highly positive sera. Two bands of 28000 and 49000 Kd were most frequently detected. Moreover, IgA and IgM specifically reacted with 49000 and 28000 Kd antigen band only. These bands were not detected in the sera of control groups. Hence, response could be of diagnostic value while discriminating the specific state of disease, particularly the antibody class in response to an antigenic fraction. The immunoglobulin IgG reactive against *M. faeni* in the sera of FLD patients were detected by immunoblotting. The IgG contained IgG1 and IgG2 which were more reactive than IgG3 and IgG4. Subsequently, the immunoblotting technique was used to analyze the antibody response against *M. feani* in the sera of farmers with extrinsic allergic alveolitis (EAA). The patients with EAA showed IgG, IgM and IgA antibody response mainly against the antigens with molecular weight of 11, 12, 25, 35 and 60 Kd. This suggested that the major antigens had a molecular weight of 11, 12, 25 and 60 Kd (Iranitalab *et al.*, 1989).

Several early reports indicated that demonstration of precipitins against offending agents provide evidence of a clinical
disease (Pepys, 1969). Multiple precipitin bands were commonly observed which showed the correlation with high intensity of disease. A few years later, doubts were expressed concerning overinterpretation of diagnostic test for precipitins (Fink et al., 1971). Subsequent studies revealed the occurrence of precipitins in sera of large number of exposed subjects with no respiratory symptoms of HP (Marx et al., 1978). These observations led to a consensus that presence of precipitins probably reflect the normal host response and is not an indication of immunological pulmonary disease.

The antibodies involved in the immune response to HP were originally thought to be of the IgG and IgM classes. Later, Parratt et al. (1975) demonstrated a significant rise in IgA response. The total IgE level were however, not elevated in farmer's lung disease. The precipitating antibodies play an important role in the diagnosis of interstitial lung disease. Microorganisms other than thermophilic actinomycetes can produce a similar condition and even among thermophilic actinomycetes species belonging to different genera can cause HP, it is therefore, important to use a battery of antigens for serological testing (Roberts et al., 1976).

The double immunodiffusion (DID) test was the first simple and reliable laboratory method for the diagnosis of farmer's lung disease. DID, however, lacks sensitivity and, therefore, requires high amount of antibody for the clinical diagnosis of HP. Two-dimensional or cross immunoelectrophoresis (CIE) offers a potential means for quantitation of precipitating antibodies directly against specific antigens within a complex antigen mixture (Treuhalt et al., 1979). Antibodies of IgG class were demonstrated by immunoblot techniques (Reese et al., 1989). Immuno-histochemical examination of pulmonary sarcoid lesion
from patient’s lung revealed localization of C3 component with deposition of immunoglobulin in the non-fibrosed granulomas. The antibodies participating in this reaction were complement fixing antibodies (Marx et al., 1987). The disease preceeded by immune complex (hypersensitivity type III) pathway where ingestion of immune complex by polymorphonuclear leukocytes and release of hydrolases mediated the tissue damage. The complement-consuming ability of the serum from the sensitized farmers against M.faeni antigen were examined by Edward (1981) through complement fixation test. In order to obtain reliable results with complement fixation test, low concentration of antigen and proper controls are necessary.

Enzyme-linked immunosorbent assay (ELISA) is yet another serological test used for the quantitation of antigen and antibodies. The test is simple, rapid and highly specific. In a study conducted by Bamdad (1980) in England, 58% of farmers exposed to thermophilic actinomycetes were ELISA positive. Almost 98% of precipitin positive sera were also found positive in ELISA test. Simultaneously, the prevalence and the titre of antibodies against T.vulgaris and M.faeni were also detected by ELISA in the farmer’s lung group from Finland (Ojenen et al., 1980). M.faeni, S.viridis, T.sacchari and T. vulgaris were tested by ELISA for the presence of antibodies in the sera of farmer’s lung patients, where as precipitin by double immunodiffusion (DID) test was simultaneously carried out by Marx et al. (1982). ELISA was found more sensitive than DID test. The ELISA absorbance values of antibodies in the serum of the farmer’s lung patient therefore, can be of diagnostic value (Hebert et al., 1985).

A few asymptomatic dairy subjects positive for precipitin against F.rectivirgula and T.vulgaris (Konishi et al., 1985) were
also having IgG antibodies as detected by ELISA. Significantly, lower IgG reactivity was found in asymptomatic and control groups. In contrast, the known subjects of extrinsic allergic alveolitis had high IgG activity against thermophilic actinomycetes. The microbial extracts from water supply of the humidifier were used as antigen to demonstrate the sensitization of the printing press workers. A significant number of workers with elevated IgG concentration were smokers as compared to non-smokers, indicating that nonsmokers have high risk of immunological sensitization. The high risk of immunological sensitization is possibly due to increased number of macrophages in the upper respiratory tract of smoker which provide a high rate of antigen clearance in smokers (Bour et al., 1992). Immune response to the purified protein antigen of *M. rectivirgula* were analyzed by immunoblotting in patients of farmer’s lung. The test revealed 20 bands of IgG and 2 bands for IgA and one band each for IgM and IgE in a highly positive serum.

Aznar et al. (1988) further applied enzyme-linked immunoelectrodiffusion assay (ELIEDA) with the same positive sera where they found only 7,10 and 8 arcs for IgG, IgA and IgM respectively. They concluded that rheumatoid factor which had high titre in the patient interfere with ELIEDA but not in immunoblotting test, which can determine the response of one particular immunoglobulin class to an antigen fraction. Immunoglobulin against *T.fusca, T.alba and T.curvata* were detected by dot-ELISA in some of mushroom worker’s lung patients. (Bogart et al., 1993). They concluded that dot-ELISA can be used to determine different classes of antibodies at a time in the same sera whereas quantitative ELISA can detect only one class of antibodies at one time. Therefore, they noted that 20% of
dot ELISA positive serum were found negative in quantitative ELISA.

The first instant response of the immune system against allergens is the elevation in IgG antibody level in susceptible individuals. Greater IgG titre against *F. rectivirgula* was reported in symptomatic dairy farm workers than asymptomatic and control as detected by indirect ELISA (Gangwar *et al.*, 1991). Similar investigation were carried out by Khan *et al.* (1995) where they found IgG against *F. rectivirgula* and *T. sacchari* in the sera of sugar mill workers. Cornelia *et al.* (1996) demonstrated specific binding of farmer's lung patient's IgG2 to *S. rectivirgula* purified antigens, whereas, no such antibodies were found in exposed and unaffected subjects. Thus subclass of immunoglobulin IgG2 reaction with *S. rectivirgula* antigens were useful in the serological diagnosis of patients with farmer's lung disease and for the isolation of disease causing antigens.