Review of literature
II REVIEW OF LITERATURE

Members of the family mycobacteriaceae can be isolated from freshwater, estuarine and marine environment as well as from the intestine of warm blooded animals. Some species are pathogenic to aquatic animals while some species comprise the ectocommensal flora of finfish, shellfish, frogs, seals etc. and some others participate in recycling organic matter (Aronson, 1926).

Isolation of mycobacteria from wild fishes

The presence acid-fast bacteria have been observed by all the investigators who have described the cases of piscine tuberculosis in different fish genera. *M. marinum*, *M. fortuitum* and *M. chelonei* are the most common among the bacterial species described in connection with mycobacteriosis in fish. Bataillon et al., (1897) were the first to isolate *Mycobacterium sp.* from pond reared carp. Besse (1949) was the first to isolate *M. anbanti* which is more identical with *M. marinum* from infected paradise fish, *Macropodus opercularis*.

In California, Hedrick, McDowell and Groff (1987) isolated *M. marinum* from striped bass. *M. fortuitum* was isolated from tropical fish, *Hyphessobrycon innesi* by Ross and Brancato (1959) and Beckwith and Malsberger (1980). A new species *M. salmoniphilum* isolated from infected salmonid fishes has been attributed as the causal organism by Ross (1960). *M. chelonei subsp. piscarium* was isolated from five locations in the states of Oregon and Montana by Arakawa and Fryer (1984).


Murichilano et al. (1986) histopathologically evaluated the gross lesions excised from North Atlantic marine fishes. As it is difficult to characterise the isolated strain up to the species level, the *Mycobacterium* sp. has been said to be isolated from yellow tails (Kusuda et al., 1987); naturally infected cod from Danish coastal waters (Dalsgaard et
al., 1992); wild caught salmons (Dixon et al., 1992); and from European sea bass (Colorni, 1992; Knibb et al., 1993). In Japan Hatai et al., (1993) isolated a photochromogenic species of mycobacterium from pejerrey, *Odontesthes bonariensis* with or without saprolegniasis.

Mycobacteria has been isolated from both healthy and infected fishes. It has been isolated successfully from snake heads (Bozzitta et al., 1995; Tortoli et al., 1996; Adams et al., 1992) and Siamese fighting fish (Pungkachonboon et al., 1990, 1992; Bozzitta et al., 1995; Adams et al., 1997). Bozzitta et al., (1995) was the first to report *M.gordonae* as the causative agent of fish tuberculosis. Through biochemical methods they identified *M.fortuitum* subsp. *acetamdolyticum* and *M.marinum*.

**Mycobacteria from cultured fishes**

Workers have isolated mycobacteria from cultured fishes of different genera at any stage during their culture in healthy or in diseased condition. It is reported that from hatchery confined Chinook salmon, *Oncorhynchus tshawytcha* (Ashburner 1977) and from different species of captive fishes (Colorni et al., 1996) *M marinum* has been isolated successfully. Buckman et al., (1990) noted an outbreak of panophthalmitis in the same species and the causal organism was either *Mycobacterium* sp. or *Rhodococcus* sp. In a guppy farm in South Africa, heavy mortalities have been reported for the first time by Bragg et al., (1990) and isolated *Mycobacterium* sp. from guppies and Oscars (McCormick et al., 1995).


Teska et al., (1997) isolated *M.abscessus* from infected cultured Japanese medaka, *Oryzias latipes* and the mean bacterial count ranged from 6.7X10^2 to 4.5X10^8
CFU/gm of the fish. *M.chelonaee* has been isolated from two Atlantic salmon rearing farms by Bruno et al., (1998).

**Mycobacteria in aquarium fishes**

Aronson (1926) was the first to describe the well-established species, *M.marinum* and to isolate it from tropical coral fishes in the Philadelphia aquarium. Giavenni (1982) identified the same species from 41 different species of fishes. Bernstad (1974) isolated *M.borstelense* from infected aquarium fishes. It is reported by Giavenni et.al (1980) that 97 marine topical fishes of the 17 genera were affected in the marine aquarium and isolated mycobacteria from the internal organs of the affected fishes. The factor for the death of 35% of the three spot gouramies, imported from an ornamental fish farm in Columbia was identified as AlB by Santacana e et al., (1982).

Shamsudin et al., (1990) tested several ornamental fishes like gold fish and red eyed tetra, *Moenkauisia sanctaeofilomina* for the presence of AlB and isolated the same (Anderson et al., 1987; Dixon et al., 1992; Landsell et al., 1993). Dailloux et al., (1992) reported that *M.marinum, M.kansasii* and *M.fortuitum* are the most frequently found species in the aquaria.

In Thailand acid-fast bacterial isolation from ornamental fishes was isolated by Chen, Adams and Richards (1997) Dixon et al., (1992) used profiles of biochemical growth characteristics to identify the Mycobacterium sp. From salmons and aquarium fishes up to species level.

**Isolation of mycobacteria from crustaceans**

Mycobacterial infection and its isolation was reported in prawns and crayfishes. Lightner and Redman (1986) reported such a case in white shrimp, *Penaeus vannamei* and from the same species, Mohney et al., (1998) isolated *M.peregrinum*. Brock et al., (1986) and Lightner (1996) isolated *Mycobacterium sp.* Runyon Group II from infected cultured fresh water prawn, *Macrobrachium rosenbergii*. Owens et al., (1992) studied about the pathology of microbial diseases in tropical Australian crustacea and reported the presence of granulomatous lesions in different internal organs of dying *M.rosenbergii*. 

8
Mycobacterial species has been included as one of the potential bacterial pathogens of crayfishes, *Austropotamobius pallipes* and the infection was reported by Anderson, Feist and Polydase (1986). Thune (1994) isolated the causal agent from hind gut, haemolymph and exoskeleton of crayfishes.

**Occurrence of fish mycobacteriosis**

Fish tuberculosis (Fish mycobacteriosis) is an infectious disease caused by organisms of the genus *Mycobacterium*, resulting usually in the formation of tubercles in various organs. The term Mycobacteriosis was coined by Parisot and Wood, (1960). The disease was reported in carp, *Cyprinus carpio* for the first time (Bataillon et al., 1963).

Parisot (1958) and Vogel (1958) has given reviews on the same disease. The review on mycobacteriosis among fishes was given extensively by Amlacher (1968). Johnstone (1913) and Alexander (1913) found a spontaneous skin infection with acid-fast bacteria in cod. In 1910, Von Betegh reported the disease in marine fish, subsequently it was observed that fishes of fresh, salt and brackish waters, aquarium and hatcheries are susceptible to the disease (Parisot and Wood, 1966).

Many acid-fast bacteria have been recorded as the aetiological agents causing mycobacteriosis in fish; in which *M. marinum*, *M. fortuitum* and *M. chelonei* are the important ones. *M. marinum* was isolated by Aronson (1926) from tropical coral fish in Philadelphia aquarium. It was found to be infecting both marine and freshwater fishes (Giavenni, 1980; Van Duijn, 1981). *M. fortuitum* was another acid-fast bacillus repeatedly found from diseased neon tetra, *Parocheirodon innesi* in 1953, although the taxonomic identification was later described by Ross and Brancato, (1959). *M. chelonei* (Arakawa and Fryer, 1984) and its subspecies *M. chelonei* subsp. *chelonei* and *M. chelonei* subsp. *abscessus* also form a major group of causal agents.

Prevalence of mycobacteriosis may seen as high as 15% in some fishes.(Parisot and Wood, 1970) and as high as 100% under extensive culture conditions (Smith, 1996). Fryer and Rohovec, (1984) and Strunjak-Perovic et al., (1995) reviewed the principal bacterial diseases among cultured marine fishes and included mycobacteriosis among them. Fish tuberculosis has been diagnosed both in
fresh water (Conroy, 1966; Majeed, Gopinath and Jolly, 1981; Bragg et al., 1990; Chinabut et al., 1990) and sea water fishes (Kusuda et al., 1987; McKenzie, 1988). High percentages of infection have been found among fishes in their natural habitats (Hastings et al., 1982).

Fish maintained in aquaria will show a higher incidence of this disease than cultured or wild species, as aquarium fishes are often kept for long periods of time under captivity compared with fish raised for commercial purposes. Aronson, (1926), Besse (1949), Nigrelli and Vogel (1968), Giavenni et al., (1980) have reported the disease in a variety of aquarium fishes. The incidence of mycobacteriosis in aquarium fish have been reported to vary from 10 to 22% (Wolke and Stroud, 1978; Santacana et al., 1982). The prevalence of infected fish in natural populations vary from 10 to 100% (Abernathy and Lund, 1978; Sakanari et al., 1983; Hedrick et al., 1987; Lawhavinit et al., 1988; McKenzie, 1988). There appears to be no bias towards the sex of the fish in the prevalence of mycobacteriosis, but the severity of the infection is apparently related to age (Abernathy and Lund, 1978; McKenzie, 1988).

The occurrence of mycobacteriosis resulted in heavy losses due to mass mortalities (Hedrick et al., 1987; Lawhavinit et al., 1988; Bragg et al., 1990). Epizootics have been reported in yellow perch by Kusuda et al., (1987) and Daoust et al., (1989) from two lakes in Alberta. 150 species of marine and fresh water fishes of more than 40 families are found to be infected (Nigrelli and Vogel, 1963).

The first report of the disease in the pond reared carp is by Bataillon et al., (1897) and in cod is by Alexander (1913), Johnstone (1913), later by Dalsgaard et al., (1990) from Danish coastal waters. Besse (1949) and Csaba (1982) recorded a massive outbreak of mycobacteriosis in paradise fish, Macropodus opercularis. In three spot gouramies, pearl gouramies and siamese fighting fish, the disease has been reported (Besse, 1952; Reichenbach-Klinke, 1954; Sato, 1962; Nigrelli and Vogel 1963; Conroy and Valdez 1964; and Conroy 1964, 1965).

mycobacteriosis in Atlantic mackerel and a preliminary report on the same was given by Bucke (1980); Hastings et al., (1982) and Marchalano et al., (1986); Aronson (1926). Sakanari et al., (1983) studied about mycobacteriosis in striped bass, Morone saxatilis from Central California and Coosbay. In Eilat, a systemic infection was recorded by Colon (1992) in the European sea bass, Dicentrarchus labrax and the prevalence of infection was 100% in the infected tanks.

In Sebastes sp. The description of the disease was given by Moser and Sakanari (1986) and in yellow perch by Kusuda et al., (1987); Daoust et al., (1989). Reports have also come on mycobacteriosis in goldfish and red-eyed tetras (Anderson et al., 1987; Shamsudin, 1990). Mycobacterial infection has been recorded in halibut, Hippoglossus hippoglossus by Sutherland, (1922); in pejerrey, Odontesthes bonariensis (Lawhavinit et al., 1988; Hatai et al., 1993) and in snake head, Channa striatus (Chinabut et al., 1990).

Mycobacteriosis is recognised as the cause of mortality in marine and fresh water fishes (Dailloux et al., 1992). Bruno et al., (1988) identified mycobacteriosis as the cause of increasing mortality on two farms rearing Atlantic salmon, Salmo salar in the Shetland Isles, Scotland.

Among crustaceans, white shrimp, Penaeus vannamei (Lightner and Redman, 1986; Mohney et al., 1998), Macrobrachium rosenbergii (Brock et al., 1986; Lightner, 1996) are reported to have mycobacterial infections. Anderson, Feist and Polydare (1986) and Thune (1994) reported the disease in cray fishes, Austropotamobius pallipes.

Mycobacteria in biofilms

Schulze—Roebbecke and Fischeder (1989, 1990) were the pioneers to study about mycobacteria in biofilms to elucidate their role in biofilms as the habitat of aquatic mycobacteria, their growth and inactivation kinetics. They observed the presence of M. kansasii and M. flavescens in the biofilm of a water distribution system and yielded $2 \times 10^5$ CFU/cm$^2$ of M. kansasii and $7 \times 10^4$ CFU/cm$^2$ of M. flavescens.

Occurrence and distribution of mycobacteria in fresh water and marine environments

Mycobacteria are ubiquitous in nature due to their potentiality to survive in any environmental conditions. Water and sediment are said to be their important sources. They are widely distributed in fresh water, marine or even estuarine habitats both in surface water and shallow sediments.

The presence of mycobacteria has been successfully proved in esturine and oceanic waters (Gruft et al., 1979), in river water (Murranzano, 1978), in lake water (Hou et al., 1983), in fresh water (Falcao et al., 1993). Viallier and Viallier (1982) investigated the modification of mycobacterial flora by the operation of nuclear power plants. Joynson (1979) found the inoculated M. kansasii was surviving in water but not in soil and he proposed that water is the natural habitat of the species. Kirchner et al., (1992) indicated the presence of mycobacteria from waters, aerosols and droplets ejected from water in acid and brown water swamps. The circulating hot water systems in hospitals (Von Reyn et al., 1994) and water polluted with industrial and domestic residues (Cordoso and Filho, 1979) are not free from mycobacteria. Kamala et al., (1994) sampled taps, wells and water coolers in different sites of a BCG trial area and strengthened their ubiquitous distribution pattern.

As sediment is very rich with organic and inorganic substances, it forms another important source of mycobacterium. Donnelly et al., (1982) reported the presence of mycobacteria from landfill leachate which drains into the environment, indicating its health hazard. Estuarine sediment (Guerine Jones, 1988), soil from acid and brown water swamps (Kirchner et al., 1992), soil samples (Kamala et al., 1994; Katila e et al.,

Fresh water and marine fishes in wild, cultured or aquarium conditions are in contact with mycobacteria as they are living in the body of the organisms, and in other important sources like sediment and water. The presence of mycobacteria has been reported from aquaria (Aronson, 1926; Bernstad, 1924; Giavanni, 1980, 1982; Santacana, 1982; Shamsudin, 1990; Dixon et al., 1992; Lansdell et al., 1993) from wild fishes (Buck, 1980; Daoust et al., 1989; Dalsgaard et al., 1992; Dixon et al., 1992; Coloni 1992; Knibb et al., 1993; Hatai et al., 1993; Bozzitta et al., 1995; Tortoli et al., 1996; Adams et al., 1997) and from cultured fishes (Ashburner, 1977; Buckman 1990; Csaba et al., 1982; Bragg et al., 1990; McCormick et al., 1995; Zhang Jongjca, 1991; Tortoli et al., 1996). Schulze Roebbecke and Fischeder (1989, 1990) reported the presence and importance in biofilms also. Shrimps (Lightner and Redman 1986, Mohney et al., 1998), crayfishes (Anderson et al., 1986; Thune 1994) and even seaweeds (Kazda et al., 1990) are reported to be inhabited by the organism.

**Mycobacteria from estuaries and ocean waters**

Few attempts have been made to recover mycobacteria from sea water as they already have been isolated from marine animals. Apart from those Viallier (1967) and Viallir and Viallier (1973, 1975, 1977) who had examined 791 samples taken off the coast of France and found mycobacteria in 176 of them. They report all strains except MAIS complex, *M.kansasii* and *M.xenopii*. During their study in 1973, they reported the isolation of *M.marinum*.

Falkinham et al., 1978, 1981) recovered MAIS bacilli from coastal waters of the southeastern part of the United States. Gruft et al., 1979 tested 38 subsurface and microlayer samples of estuaries and ocean waters, 16 of which yielded a total of 30 strains. 19 strains were MAIS organisms. *M.gordonae* and *M.terre* were also present and they postulated the sources of *M.intracellulare* and *M.scrophulaceum*.

In United States, Gruft et al., (1981) examined 520 samples from a larger area of the southeastern seaboard and found MAIS bacilli in 128 of them, including
M. gordonae and M. terre in 191 samples. In New Zealand, Kazda et al., (1990) identified slow growing scotochromogenic mycobacteria from surface waters and sphagnum vegetation. The organism was identified as M. cookii sp. nov. From northern Brook waters, mycobacterial strains were isolated by Livanainen et al., (1997).

**Mycobacteria in piped, polluted and treated waters**

Report on the presence of acid-fast organisms in slime and scrapings from inner side of water pipes was given by Brem (1909) and Bertzke (1910). Kubica et al., (1961, 1963) and Paull (1969) were trying to find out the possible sources of atypical mycobacteria in environmental material. A variety of scotochromogen and rapidly growing mycobacteria were recovered by Paull (1969) from four of thirty samples of tap water and four of nine colliery shower heads. A variety of fast growing mycobacteria were isolated from a water tank by Stanford and Beck (1969) and were designated as M. friedmannii. Bailey et al., (1970) isolated M. kansasi from tap water in USA. In an ensuing investigation Bullin et al., (1970) recovered 47 strains scotochromogens and were found randomly distributed in the samples from three hospitals, subsequently it was noted that M. xenopi occurred predominantly in the hot water faucets.

McSwiggin and Collins (1974) examined water supplies in one hospital in London and obtained 18 strains of M. kansasi and six strains of M. xenopi from 65 water taps. However neither organism was recovered from water samples taken from an outside tap connected directly with the mains supply.

In Winnipeg, Manias and Vanbackethout (1976) isolated 17 strains of M. kansasi from a hospital water supply. In the same year Dizon et.al found acid-fast bacilli in the tap water. The presence of M. xenopi in hot water generators and taps was reported by Gross et al., (1976). Cordoso and l'ilho (1979) isolated slow growers and potentially pathogenic mycobacteria like M. avium-intracellulare, M. scrophulaceum and M. fortuitum from water polluted with industrial and domestic residues.

Steadham (1980) recovered M. kansasi from 8 of 19 representative outlets in a town of Texas. M. gordonae was present in all 19 samples and M. fortuitum in two. Engel et.al (1980) examined water taps in Rotterdam and found M. kansasi in 38 of 78 among them during six samplings in one year. Clostrim et al., (1981) recovered M. xenopi
from tap water in New Haven and Collins et al., (1981) found scotochromogens and rapidly growing mycobacteria in a tap water of two laboratories.

In a report from Czechoslovakia, Kaustova et al. (1981) referred 510 samples from a municipal water supply, in 18 of which they found *M. kansasi*. They also examined 1589 samples of water from collieries finding scotochromogens and rapidly growing mycobacteria in 233 and *M. kansasi* in 20 samples. 12 of these strains of *M. kansasi* came from one area and all others isolated from pithead shower bath outlets. Park and Brewer (1976) isolated the organisms from pools of Tennessee. Dailloux et al., (1980) found it in swimming pools and also recovered *M. kansasi* and *M. fortuitum* from the same water.

Other mycobacteria have also been found in aquaria. Caroli et al., (1982) isolated 43 strains from 53 samples of aquarium water (19 in households and 36 in pet shops) The most frequent isolate was *M. gordonae* but potential pathogens including MAIS bacilli, *M. kansasi*, *M. chelonei* and *M. fortuitum* were found. *M. marinum* was isolated from only one sample. Various species including *M. fortuitum*, *M. chelonei*, *M. avium-intracellulare* have been isolated from zoo aquaria (Pattyn et al., 1971; Goslee and Wolinsky 1976). Collins et al., (1984) reviewed the occurrence of mycobacteria in natural, piped and treated waters. Mycobacteria were isolated frequently by Schulze-Roebbecke and Buchholtz (1992) from domestic water supplies. Katila (1995) isolated potentially pathogenic mycobacteria from surface waters in the Finnish environment. They isolated *M. fortuitum* and *M. gordonae* during next year and the findings suggest that the source of mycobacterium is water. From surface and treated waters, the organisms were isolated by Neumann et al., (1997). Kirchner et al., (1992) identified *M. avium*, *M. intracellulare*, *M. scrophulaceum* etc. from waters collected from four geographically separate aquatic environments of the south eastern United States.

*M. avium* strain was isolated by Von-Regn et al., (1994) from circulating hot water systems in two hospitals. Water taps, wells and water coolers at different sites in a BCG trial area has been investigated by Kamala et al., (1994) and found the presence of MAIS complex, *M. dienhoferi*, *M. vaccae*, *M. smegmatis* and *M. terrae*.
Isolation of Mycobacteria from sediment

Mycobacteria have been isolated from sediment and soil samples from different locations as these form important sources of non-tuberculous mycobacteria. Donnelly, Scarpino and Brunner (1982) enumerated mycobacteria and streptococci present in landfill leachate to determine the significance of this leachate when it drains into the environment. Propane utilising mycobacteria has been isolated by Hou et al., (1983) from the soil sample in the vicinity of Bayway refinery, Linden. From estuarine sediment, phenanthrene mineralising mycobacterial strain was isolated by Guerin and Jones (1988) and Guerin and Jo (1986). Lee and Lee (1991) reported the presence of mycobacterium among marine heterotrophs during their study about the seasonal distribution in sediment.

Two species of mycobacteria, *M.chelonei* and *M.fortuitum* have been isolated by Owens et al., (1992) from the gravel of culture ponds of *Penaeus esculentus* and *Macrobrachium rosenberghii*. Kirchner et al., (1992) isolated and identified MAIS from soils of some acid and brown water swamps. MAIS complex, *M.aurum*, *M.chelonei* subsp. *chelonei*, *M.diernhoferi*, *M.fortuitum*, *M.gadium* and *M.thermoresistible* were isolated by Kamala et al., (1994) from soil samples.

Procedures for mycobacterial isolation

During isolation of mycobacteria, one of the important step is decontamination of the sample which allows the maximum retrieval of mycobacteria from heterotrophs. Cordoso and Filho (1979) treated polluted water samples with 4% NaOIl and 0.34% benzalkonium chloride. Falkinham’s method of decontamination using 1%, 2% and 4% NaOIl (Brooks et al., 1984) and Engbaek’s method (Engbaek et al., 1967) using 3% sodium lauryl sulphate and 1% NaOIl are adopted by Kamala et.al for isolating mycobacteria from soil and water samples. Gangadharam’s method using 1% cetrimide (Joseph et al., 1969); two modifications of Falkinham’s method using 2% and 4% NaOIl has been employed. For water samples, Falkinham’s method with 4% and 8%NaOIl (Falkinham et al., 1980); Goslee and Wolinsky’s method with NaOH, NaOCl, 4% NaOIl and 4% H2SO4 (Goslee and Wolinsky, 1976) or Engel’s method (Engel et al., 1980) with 3% SLS and 1% NaOIl are also used by Kamala et al., (1994).
Decontaminants like 0.7 mol/l NaOH followed by 50 gm/l oxalic acid and 0.9 mol/l H1 Sub (2) SO Sub (4) combined with 0.5 g/l cycloheximide are used by Livsnainen, Martikainen and Katila (1997) for water samples. Neumann et al., (1997), compared twelve methods to isolate mycobacteria from surface and treated waters. They decontaminated surface waters with cetylpyridinium chloride (CPC) (30 min., 0.05%) firstly then with a cocktail of NaOll, cycloheximide and malachite green after preincubation of the sample in Tryptic soy broth (TSB). They used CPC 0.005%, 0.05% (30min) as decontaminants for treated waters.

Even though the methods employed for isolation of mycobacteria from water and sediment samples can also be used to process fish samples Dalsgaard et al., (1992) and Dixon et al., (1992) specified some methods for successful isolation of the organism. Dalsgaard et al., (1992) used NaOll and oxalic acid as decontaminants but Dixon et al., (1992) used 2%HCl or 4% NaOll.

Teska et al., (1997) followed three isolation procedures from whole fish homogenates and obtained highest isolation rates on submerging whole fish in individual bags of modified broth at 1:10 (weight/volume) dilution for one hour, homogenising and plating on solid media.

Media used for isolation

Workers have used different synthetic media in different combinations during the isolation procedure of mycobacteria and they have their own choice to select the media, ensuring the availability of nutrients for the growth of the organism in required quantity. Most popular among the group of usually using media is Loewenstein Jensen (LJ), an egg based medium and is widely used (Brock et al., 1986, Dalsgaard et al., 1992, Kamala et al., 1994, Neumann et al., 1997). Blood agar plates (BAP) (Brock et al., 1986), Petragnani's medium and Brain Heart Infusion agar (Hedrick et al., 1987) have been used in some cases.

A group of agar based Middlebrook series like 7H10, 7H9, 7H11 etc. have been used widely with or without adding supplements. Kamala et al.,(1994) used Middlebrook 7H11 agar and Falkinham's selective medium.
Teska et al., (1997) used modified 7H10 with albumin, dextrose and catalase (ADC) enrichment to isolate mycobacteria from whole fish homogenates. Middlebrook 7H11 with OADC supplement, glycerol egg medium and pyruvate egg medium has been used by livanainen, Matrikainen and Katila (1997) to compare the degree of isolation of mycobacteria from Brook waters. Pungkachonboon et al., (1992) isolated rapidly growing photochromogenic mycobacteria from Siamese fighting fish on Ogawa egg medium and its modified forms like Ogawa Egg Yolk medium (OEY) and Ogawa whole egg medium containing afoxacin and ethambutol (OEOE) have been used by Neumann et al., (1997). Chen, Adams and Richards (1997) used Long’s medium, Engel’s minimal essential medium, Sauton’s medium and modified Sauton’s medium for producing extra cellular products from Mycobacterium spp. Pungkachonboon et al., (1990) mentioned the incubation temperature for the samples as 28°C. Colorni (1992) and Neumann et al., (1997) were done the incubation at 24°C and 37°C respectively.

**Antibiotic activity of some natural products against mycobacteria.**

The inhibitory activity of the aqueous and alcoholic extracts of *Rhizophora mangle* L. are reported by Rojas- Hernandez and Coto Perez (1978) and found the susceptible nature of mycobacterium strains. They mentioned the minimum inhibitory concentration of the extract for the activity. Organic solvents and hot water extracts of 100 marine microalgae were screened by Miura and Matsunaga (1989) for their antibiotic activity against *M. phlei* using the paper disc method and noticed that organic solvent extracts of 33 strains had activity against the species. Using the same method, Murakami et al., (1984) examined the activity of planktonic organisms. The distinct and widespread activity of the ether extract of Asterionella japonica towards *M. smegmatis* was reported by them.

As streptomycetes form an active source of efficient antimicrobials, Chandramohan and nair (1991) isolated them from sediments of andaman and Nicobar islands to study their antagonistic property against mycobacteria. Lohsiri et al., (1994) proved antimicrobial capability of the extract of marine sponges against *M. smegmatis*. Invitro antibacterial activity of massetolides A-II (1-8) and viscosin , isolated from two species of pseudomonas against *M. tuberculosis* and *M. avium- intracellulare* was reported by Gerand et al., (1997).
Biodegradation of some natural products by mycobacteria

Mycobacteria are well known for their extraordinary capability for degrading natural compounds. Guerin and Jones (1988) isolated mycobacteria capable of utilising phenanthrene. Compounds like 1- nitropyrene from oil contaminated sediments (Hleitkamp et al., 1991), PAH in a pristine ecosystem (Hleitkamp and Cemiglia, 1989; Spic et al., 1997), ground water pollutant mixtures like acetone, cyclohexane, styrene, benzene, ethylbenzene, propylbenzene, dioxane, 1-2 dichloroethane (Burback and Perry, 1993) are found to be degraded powerfully by different species of mycobacteria including M.vaccae (Burback and perry, 1993). Sepic et al., (1997) proposed a floranthene biodegradation pathway. Growth of Mycobacteria on Carbon Monoxide and Methanol was studied by Park et al., 2003.

Molecular studies on Mycobacteria

Knibb et al., (1993) evaluated PCR as a diagnostic tool for mycobacteriosis and found the method as specific and most sensitive. The method is facilitating the screening of samples in the field as well as the new stocks for latent infections. Bruno et al., (1998) adopted PCR technique for the complete identification of M.chelonei isolated from moribund atlantic salmons. McCormick, Il Hughes and McLoughlin (1995) amplified 16S rRNA gene sequences through direct gene sequencing of polymerase chain reaction and it is used to identify rapidly growing, acid-fast organism isolated from a cichlid oscar. A 924 bp DNA fragment of 16s rRNA was amplified through PCR technology of Talaat et al., (1997) and rapidly identified M.marinum, M.fortuitum and M.chelonei in fish. This report yielded unique restriction patterns for each mycobacterial species infecting fish to the species to the species level. Assessment of genetic diversity is important in epidemiological studies of nontuberculous mycobacteria (NTM), as data from these studies could be used to monitor trends in the occurrence of new strains, identify possible sources of infection, and differentiate individual strains (Tenover et al.,1997).Knibb et al., (1992) developed a PCR technique to identify the pathogen of mycobacteriosis in european sea bass without sacrificing the animals. The method is by direct sequencing and analysis of approximately 600 bp of the rDNA. The sensitivity of the method allows the culturist to remove the asymptomatic fishes from the cultural conditions. Knibb et.al (1993) identified M.marinum by the same method as before.
Two toluene degrading strains (T103 and T104) were studied by Tay et al., (1998) and identified their similarity with *M. aurum* and *M. komossense* from 16S rDNA sequences. Vanitha et al.,(2003) reported Large-Restriction-Fragment Polymorphism Analysis of *Mycobacterium chelonae* and *Mycobacterium terrae* Isolates.

Thorel et al., (1998) reported the isolation and identification of *M. bovis* from infected zoo animals by epidemiological study using genetic markers such as IS-6110 based DNA finger printing system and they differentiated *M. bovis* strains, some strains presented a single copy but multiple copies by others. Arakawa and Fryer (1984) adopted biological, physiological genetic and mycolic acid properties for the taxonomic analysis of mycobacteria, isolated from salmonid fishes. From a percent guanine plus cytosine value of 63 plus or minus 1.7 %, they confirmed the isolates as the genus mycobacterium. Shamsudin et al., (1990) also investigated guanine plus cytosine percent value in mycobacteria isolated from infected ornamental fishes; but the strain was not identified up to the species level.