

## Molecular Biology: A Field of Great Contribution in Paratuberculosis Research

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### Introduction

With advances of molecular biology, it has been possible to study the structural components of *Mycobacterium avium* subsp. paratuberculosis (Map) which is the main causative agent of John's disease in cattle and other ruminants. Molecular Biology also paves the way to differentiate between Map from other closely related species of mycobacterium origin. Organism was first explored by John and Frothingham in the year 1895. The prime site of invasion of the organisms is intestinal macrophages in the vicinity of Peyer's patches, where it can persist for a considerable long periods (Momotani et al., 1988). These lead to an appropriate T- cell response, which is characterized by release of interferon gamma (IFN-g) and other cytokines for examples interleukin-1a (IL-1a) IL-6 and IL-2 (Coussens, et al., 1999). It is also evident that at the edge of infection, there is a type-2 like response, which is characterized by immunoglobulin G1 (Stable, 1998). So, molecular biology has opened a door to study various significant pathogenic structure of Map, which ultimately can keep a significant impact in the field of paratuberculosis diagnosis and has been instrumental in the development of specific and sensitive diagnosis tests.

### Identification of Immunogenic Components of MAP

Various immuno - pathogenic particles of Hap have been studied which can help in the development of sero-diagnosis kits against paratuberculosis. Various cell wall components of Map especially glycopeptidolipide and lipoarabino-mannan (LAM) has been extensively studied due their potent. Pathogenic property (K. Stevenson and J. M. Sharp, 1997). Several molecular technique like crossed immunoelectro-phoresis (CIE), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting has become

successful to identify various mycobacterium species specific proteins (Bech-Nielsen et al., 1985; Valentine-Weigand et al., 1992 and Deshpande et al., 1992). For example a protein 42 kDa which was identified as a potent immunogenic to Map; but not in case of *M. bovis* (White et al., 1994).

Another approach to species specific gene identification is monoclonal antibody production technique, which has been undertaken primarily to provide diagnostic tools for Map infection in humans (Reviewed by K. Stevenson and J.M. Sharp, 1997). Other important advance that has improved quick detection and differentiation of various mycobacterium species is Igt11 expression system pioneered by Young et al. 1985; which can identify specific gene of a specific mycobacterium group including Map.

### Identification of Genetic Elements of MAP

Molecular techniques revealed various genetic elements of Map like rRNA genes (16S, 23S and 5S) as well as spacer regions which are highly conserved among Map (Rogall et al., 1990a). The foremost genetic molecules of Map discovered was an insertion element that is IS900 (Green and Collins et al., 1989). These attribution help to development of new diagnostic tests which are based on polymerase chain reaction (PCR).

Poupart et al. (1993) also isolated a DNA fragment, F57, which is potentially subspecies specific for Map.

### IS900 PCR

I dex laboratorjes, Inc., first developed IS900 PCR based diagnostic kit for detection of paratuberculosis. Cousins et al. demonstrated that these IS900 PCR can give false-positive results in all mycobacterium species except Map. Using these method it is also possible to amplify Map strain of AIDS patients (Millar et al., 1995).

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#### Differentiation of Various Strains of MAP

Using various molecular techniques like-restriction fragment length polymorphisms (RFLPs), conventional restriction endonuclease analysis and pulsed-field gel electrophoresis (PFGE) could discriminate various species of mycobacterium from Map, as well as differentiate various strains of Map species. It does help to identify pathogenic strains of Map, which are the causative agent of disease. Recently other molecular techniques has been developed to differentiate strain variation like-Multilocus enzyme electrophoresis (MEE); which has advantage over RFLP and PFGE is that less DNA is required and it is rapid and more sensitive (Lee et al., 1994).

#### Future role of Molecular Biology in Paratuberculosis Research

Due to sluggish growth and present of various anatomical barriers like - waxy coat and complex cell wall in the structure of Map, make a difficult task for researchers to do various genetic manipulation, which can be done very easily compared to other bacterial species. Genetic manipulation is an important instrumentation, by which a researcher can detect the potentiality of an expressed protein or antigen. Though molecular biology has open door to detect various immuno-pathogenic components of Map, but the role of these proteins and genes in Map infection is still infant.

In the past few years, there are various progress has been attribute to the development of genetic manipulation system in Map, which has paved the way for exploring the effect of various virulence components of Map. In future it may be possible to induce the virulence factor of the organism directly to the host or by coating with

various non-particles to induce safe and stronger immunity to the host and ultimately it will play an immense role in development of new generation vaccines which will be more powerful and effective to alleviate the deadly disease from animal population.

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## NEWS

A technical seminar on " The Diversity of Veterinary Profession" was organised by Gujarat Veterinary Council at Gandhinagar, Gujarat on World Veterinary Day. There were approx. 300 veterinarians participated in that seminar. Speakers has given lecture on various opportunity and Diversification in Veterinary Profession.