Short Communication

In-Vitro cultivation of mycobacterium leprae and confirmation by molecular method

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Received Date: 21/05/2016 Revised Date: 19/06/2016 Accepted Date: 11/07/2016

Access this article online

Quick Response Code:

Website: www.statperson.com

Volume 6
Issue 3

INTRODUCTION

Mycobacterium leprae was the first organism described as the causative agent of an infection. It was described by Dr. G. Armaner Hansen as the causative agent of leprosy in 1874. Thereafter, numerous agents/bacteria have been described as causative agents of disease like Mycobacterium tuberculosis, Vibrio cholera, etc. Almost all bacteria discovered till now have been grown in artificial culture in chemically defined media (in-vitro cultivation) in the laboratory. However, Mycobacterium leprae though first described as a causative agent still remains uncultivable in the laboratory in artificial culture media. Numerous attempts have been made by different workers to grow this organism in the laboratory but all attempts at cultivation have been unsuccessful till date. As it would be too extensive to list all the unsuccessful attempts at cultivating this organism on artificial culture media, only a few important references are cited here (see References 1-10 below). The International Leprosy Congress 1978 concluded that "so far there is no proof that a genuine culture of the leprosy bacilli has been obtained". This position holds good even today. An in-vitro cultivation method for Mycobacterium leprae is described here. The artificial culture method developed by us consists of a biphasic media (solid and liquid phase) which is hereafter referred to as the "PG Media" after the name of the workers. Tissue taken from the ear lobule or from the lesions of lepromatous leprosy patients which were positive on smear examination, and graded 4+ to 6+ as per standard bacillary grading classification, were inoculated into the PG media in duplicate after treating the tissue with antibiotic and antifungal solution. The incubation was done in duplicate at 18C-20C and also at 37C. Incubation of the media was carried out in a slightly slanting position so that the liquid portion covers the solid portion of the media. Incubation was carried out for a total period of 12-16 weeks before declaring the culture as negative. It was observed that solidly stained acid fast bacilli as stained by modified Ziehl–Neelsen's stain resembling Mycobacterium leprae appeared in the liquid phase at about one month's time. Thereafter, colonies started to appear on the solid phase at about 6–8 weeks time gradually attaining full size at about 12-16 weeks. Colonies appeared only in the PG Media incubated in the B.O.D incubator at 18C – 22C. No colony formation was observed at 37C even after 12-16 weeks. The colonies that appeared in the PG media in the B.O.D. incubator were 2-3mm pearlywhite in colour, occasionally with a buff centre and were rough. They were non-emulsifiable in Pg: 2 nature. The composition of the Biphasic PG Media is under Patents Act, 1970. Mycobacterium Leprae was grown in PG Media developed by us, were sent to National JALMA Institute for leprosy and other mycobacterial diseases, ICMR, Agra, INDIA, where it was confirmed as Mycobacterium Leprae by molecular method. The amplified gene is a repetitive sequence of Mycobacterium Leprae and is 100% specific to the species. No other species of Mycobacterium have this repeat in their genome. The amplified gene is 129 Base pair. The cultured species is also confirmed by

How to cite this article: D Pal, K Ghosh. *In-Vitro* cultivation of mycobacterium leprae and confirmation by molecular method. *International Journal of Recent Trends in Science and Technology* July to September 2016; 6(3): 67-68 http://www.statperson.com

amplification of short tandem repeat. This simple in-vitro cultivation technique of Mycobacterium Leprae may now open up a new era of production of specific monoclonal antibody, antibiotic sensitivity testing, new vaccine against Leprosy and many other research work which will help to eradicate Leprosy from the world.

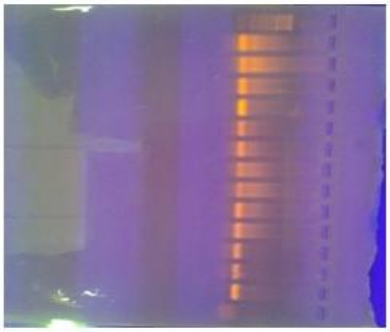

Figure 1

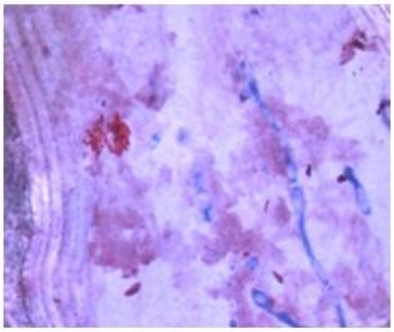

Figure 2

Legend

Figure 1: (Molecular confirmation of Mycobacterium Leprae) {By courtesy :JALMA,Agra}

Figure 2: (Modified Z-Nstain from culture of M.Leprae showing globi)

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Source of Support: None Declared
Conflict of Interest: None Declared