Advanced Journal of Microbiology Research Vol. 2013
Available online at http://internationalscholarsjournals.org/journal/ajmr

Short Communication

Differentiation of parasitic and saprophytic leptospirae by oxidase test – A hasty modus operandi

Nagarajan Prabhu[1]*, Sivasamy Senthil Prabhu[1], Mohammed Thanveer[1], Nagarajan Revathi[1], Narayanan Josna[1], Rajaputhran Darsana[1], Danialas Joseph Pushpa Innocent[2] and Palaniswamy Chinnaswamy[3]

[1]Postgraduate and Research Department of Microbiology, Arts and Science College, An Institution of Kovai Medical Center and Hospital, Coimbatore, India.

[2]Division of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, India.

[3]Institute of laboratory Medicine, Kovai Medical Center and Hospital (KMCH), Coimbatore, India.

Accepted 14 October, 2012

The use of the cytochrome oxidase test reagent, as a differential agent to study the pathogenic and saprophytic leptospires was studied. Growth of the leptospiral strains of the saprophytic group was almost involved in the conversion of colorless tetramethyl p-phenelene diamine dihydrochloride solution to dark brown color whereas pathogenic leptospirae strains showed reddish brown color. Saprophytic strains required 20-35 min for oxidation of cytochrome, however, pathogenic strains required more time, about 45 min for cytocrome oxidation. By this preliminary investigation, it is now possible to differentiate and report the saprophytic and parasitic strains based on the differential observation of the cytochrome oxidase test.

Key words: Pathogenic, leptopsiral strains, saprophytic strain, 1% oxidase reagent, rapid detection.

INTRODUCTION

For many years rats and dogs were considered to be the primary animal carriers, but as the search for leptospires continues the host range broadens not only among domestic animals but in variety of wild, feral animals and humans (Galton, 1957). Leptospirosis is considering as a major public health problem that affect the kidneys and may be found in the Lumina of the convoluted tubules and may be shed in the urine for long periods. Differentiation of the rapidly increasing number of the pathogenic leptospiral strains is highly important for an epidemiological and epizootiological point of view (Van Der Hoeden, 1955).

The differentiation of pathogenic and saprophytic leptospirae is involved in the practical laboratory medicine not only for the taxonomical point of view but also a major rapid diagnostic tool to process the specimens in the health care industry to treat the patients in time (Fuzi and Csoka, 1961). Due to loss of the virulence leptospirae after the culture passage, the determination of animal pathogenicity study is not reliable differentiation method. Lot of techniques was introduced to differentiate the strains of leptospirae like 8-azaguanine assay (Johnson and Rogers, 1964), egg yolk reaction test and copper sulphate test (Fuzi and Csoka, 1960). Some of the stu-dies highlighted the positive oxidase tests with patho-genic leptospiral types performing qualitatively. Some of the studies demonstrated the colonial growth of patho-genic and saprophytic leptospirae on Cox's medium by adding p- aminodimethyl-anilineoxalate to the colonies (Goldberg and Armstrong, 1958).

In this investigation, the oxidase activity to leptospirae in liquid EMJH medium (5% inactivated rabbit serum) and observed that saprophytic leptospirae reacted more rapids and strongly than pathogenic strains. After direct examination of the reaction under optimal conditions like temperature, light, suspension density and concentration of the reagent for the clear differentiation of serovar groups. This report is mainly concerned with the performance of a simple method for differentiating pathogenic and saprophytic leptospires (Yanagawa et al., 1963) and this technique is a presumptive test which can be perfor-

*Corresponding author. E-mail: prachanna_76@yahoo.co.in. Tel.: 91 – 422 – 2629367. Fax: 91 - 422 – 2629369.

Table 1. Oxidase activity of different cultures of *Leptospira.*

Rapid Oxidase producer	Time taken	Slow Oxidase producer	Time taken
Pathogenic leptospirae			
L. pomona	15 min	*L. australis*	45 min
L. grippotyphosa	15 min	*L. autumnalis*	45 min
		L. icterohaemorrhagiae	45 min
		L. canicola	45 min
		L. javanica	45 min
		L. hebdomadis	45 min
Saprophytic leptospirae			
L. semaranga	20 min	*L. andamana*	35 min
L. patoc	20 min		

med in ordinary laboratory where the lab is not equipped for the leptospiral research work (Fuzi and Csoka, 1961).

MATERIALS AND METHODS

Eight strains of pathogenic serotypes of leptospires (*Leptospira australis Leptospira autumnalis, Leptospira icterohaemorrhagiae, Leptospira pomona, Leptospira canicola, Leptospira javanica, Leptospira grippotyphosa,* and *Leptospira hebdomadis*) and three strains of saprophytic leptospires (*Leptospira semaranga, Leptospira andamana* and *Leptospira patoc*) were used in this investtigation. All the strains of leptospiral serotypes were obtained from Regional Medical Research Centre, WHO collaborating centre for diagnosis, reference, research and training in leptospirosis, Port Blair, Andaman and Nicobar islands, India.

Stock cultures of leptospires were maintained in a basal EMJH liquid medium containing 5% pooled rabbit serum, 0.02 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4) (Johnson and Harris, 1967), 10^{-3} M NH_4Cl and 5 µg/ml of thiamine (Johnson and Gary, 1963). Cultures were incubated at 30^{o}C and transferred every 7 days. 5-flurouracil (100 µg/ml) was incorporated in the basal medium to control any contamination that might have occurred (Johnson and Rogers, 1964).

To 4 ml of well developed 8 to 14 day old cultures of leptospirae growing in EMJH liquid broth in ordinary test tubes at 30^{o}C, 0.1 ml of a freshly prepared 1% N,N,N',N' tetramethyl paraphenelene diamine dihydrochloride (TPDD) solution was added and incubated at room temperature in light (Fuzi and Csoka, 1961) . Readings were made with the direct observation at various times from 10 to 60 min. The color obtained in various time duration was recorded and easily identify the pathogenic and saprophytic leptospires. The change in the colour was also recorded using uv visible spectrophotometry at the wavelength of 540 nm.

RESULTS AND DISCUSSION

All the previously recognized serotypes of pathogenic and saprophytic leptospirae have been tested by this method and gave consistent results at repeated examina-tions. The preliminary studies on the distribution of oxi-dase in leptospirae were accomplished by pouring tubes containing test leptospiral cultures with 1% TPDD. All the serotypes included in this study were capable of destroying the colorless TPDD as determined the formation of colorful compound. But the time taken for the conversion is quite different from one serogroup to others (Table 1). Colonies of *Pseudomonas aeruginosa* (positive control) destroy TPDD much more rapidly than do cul-tures of leptospirae. The listed pathogenic leptospirae showed pale reddish brown color after releasing the enzyme oxidase by the strains; cultures of saprophytic groups were dark brown to blackish brown. The time taken by the saprophytic leptospirae for the color change was from 20 to 35 min.

The maximum time taken by the pathogenic strains was 45 min. The two serotypes *Leptospira grippotyphosa* and *L. pomona* showed the results within 15 min whereas remaining serotypes included in this study took maximum of 45 min. The medium control and water control was kept for comparison. It was first demonstrated in the colonies of *Neisseria* sp. on a missed culture plates. Some studies highlighted the oxidase technique is highly applicable to distinguish pseudomonads from enteric bacteria. This is a low sensitivity method by which greater differentiation was observed among the strains of the genus. TPDD act as an electron donor where the electrons were released and transferred to cytochrome C to oxidize it and this test serve as biochemical marker for the differentiation of bacteria (Fuzi and Csoka, 1961).

The method of differentiation proposed in this paper is as simple and standard compared with other biochemical methods. The usage of 1% TPDD resulted in a clear distinction between all saprophytic and pathogenic leptospires tested so far in the laboratory. Thus, by using a small inoculum with TPDD reagent, the difference in cytochrome release between the pathogenic and saprophytic leptospires is maximal.

REFERENCES

Fuzi M, Csoka R (1960). Differentiation of pathogenic and saprophytic leptospirae by means of a copper sulphate test. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. 179: 231- 237.

Fuzi M, Csoka R (1961). Rapid method for the differentiation of parasitic and saprophytic leptospirae. J. Bacteriol. 81: 430-432.

Galton MM, Powers DK, Hall AM, Cornell RG (1957). A rapid microscopic-slide screening test for the serodiagnosis of leptospirosis. Am. J. Vet. Res. 19: 505-512.

Goldberg, Armstrong (1958). Colonial growth of pathogenic and saprophytic leptospirae on Cox's medium. J. Bacteriol. 77: 512- 513.

Johnson RC, Gary ND (1963). Nutrition of Leptospira Pomona II. Fatty acid requirements. J. Bacteriol. 85: 976-982.

Johnson RC, Harris VG (1967). Differentiation of pathogenic and saprophytic leptospires. Growth at low temperatures. J. Bacteriol. 94: 27-31.

Johnson RC, Rogers P (1964). 5- flurouracil as a selective agent for growth of leptospires. J. Bacteriol. 87: 422- 526.

Van Der Hoeden J (1955). Epizootiology of leptospirosis (canicola) in the bovine and other species of Israel. J. Am. Vet. Med. Ass. 126: 207-210.

Yanagawa R, Hiramune T, Fujita J (1963). Effects of Carbon dioxide on the growth of the colonial growth of pathogenic leptospirae. J. Bacteriol. 85: 875-880.