GROWTH CHARACTERS AND ANTIMICROBIAL SUSCEPTIBILITY OF LEPROSY-DERIVED MYCELIAL ORGANISM

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Growth characters and anti-microbial susceptibility of 36 leprosy-derived mycelial isolates resembling Dermatophilus species have been studied. The organism gave actinomycetoid type of growth on CMY-PD agar. In liquid medium, with similar constituents, the growth consisted of turbidity in the medium with a sheath on the top and mucoid deposit at the bottom. All the 36 isolates tested were sensitive to Rifampicin and Oflaxicin. 12 isolates showed resistance to DDS.

Key Words : Leprosy derived organism, growth characters, susceptibility.

Introduction

Leprosy organism has not been grown in its mycobacterial form to the satisfaction of scientists. Chatterjee in 1976[1] reported cultivable coccoid precursors. Chakraborty (1987)[2] claimed cultivation of a chemo-autotroph in supplemented minimal basal medium. Bhatia (1988)[3] demonstrated proliferation of filaments parallel to increase in AFB with typical M.leprae morphology in cold cultures. Bhatia and Thawani (1994)[4] reported cysts and oval cells from cultures in biphasic medium. Subsequently we have isolated a mycelial organism from leprosy material in biphasic and solid media. The organism resembled Dermatophilus species (Bhatia 1994)[5]. The present note deals with growth characters of the organism in solid and liquid media and antimicrobial susceptibility of isolates by drug-incorporation and disc diffusion methods.

Materials and Methods

The material consisted of (a) biopsy suspensions from multibacillary leprosy patients with high BI (Ridley grade 3 or more) treated with MDT for varying period (b) m.f.p. harvests and (c) skin scrapes and tissue fluid samples collected from MB leprosy lesions. The details for preparing suspensions are described in one of our previous papers (Bhatia 1988)[3]

A biphasic medium was used for the study. The slope was made of CMY-PD agar (Corn meal 20 g, Yeast 4 g, Peptone 10 g, Dextrose 10g and agar 20g in one litre distilled water). The overlay consisted of Earle's balanced salt solution without $NaHCO_3$. The method for preparation and sterilization is already described in a previous publication (Bhatia and Rathinavell 1992).[6]

The tubes were inoculated with all the sterility precautions. The cultures were incubated at 37°C for 5 days. Colony character, wet preparations and ZN-stained smears were studied. Fermentation reactions, production of catalse, urease, hydrolysis of gelatin, casien, xanthine and tyrosine (Cruikshank, et al 1975,[7] Baron and Finegold 1990[8]) and pathogenicity in scarified rabbit skin were tested.

The subcultures from the original cultures were made in (i) solid CMY-PD agar, (ii) Biphasic medium with slope of CMY-PD agar and (iii) Earle's B.S.S. with and without 0.5% CMY-PD agar. The inhibitory effect of DDS, Rifampicin and Oflaxicin was studied by incorporating drug in CMY-PD agar in different concentrations (100 to 0.0001 mg

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per ml) and also by disc diffusion method using 2 mcg, 5 mcg and 100 mcg (per disc) concentration of Oflaxicin, Rifampicin and DDS respectively.

Results

Most of the preserved and fresh samples including biopsies, mouse foot pad harvests and skin scrapes (or tissue fluid) from lepromatous lesions gave positive growth on biphasic medium (Table I).

Table I. Isolates obtained from leprosy material using biphasic medium

Mateial	No.	No. positive
Biopsies	12	12
Mouse foot pad Harvests	13	12
Scrapes/tissue fluid	15	12

Microscopically the growth consisted of dark masses, cystic structures, oval cells, bacillary bodies, motile & non-motile spores, round bodies and hyphae. Biochemical reactions for these isolates are shown in Table II.

The isolates produced varying degree of inflammatory reaction in scarified rabbit skin. Some of the isolates produced well defined exudative lesions leading to crust formation (Fig 1).

On biphasic and on liquid medium the

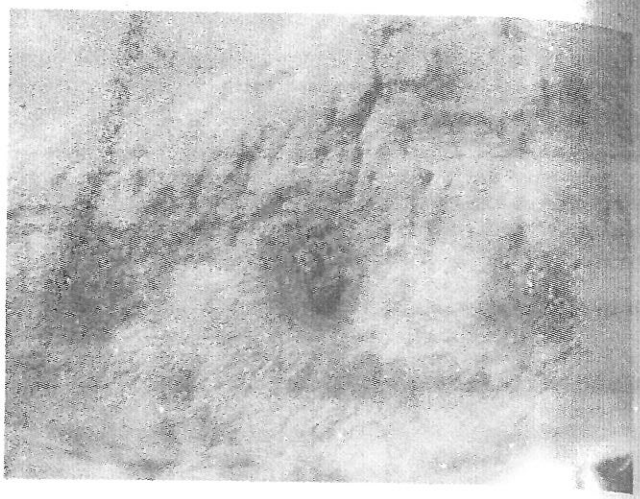

Fig. 1. Animal pathogenicity of three isolates in scarified rabbit skin. The degree varies with different isolates.

growth consisted of surface scum with deposit at bottom and turbidity in the liquid portion (Fig 2).

The isolates from biphasic medium could be subcultured on Nutrient agar and CMY-PD agar slopes in tubes or bottles. The colonies appeared within 3-5 days. On keeping the tubes, the colonies grew further resulting in a brown coloured actinomycetoid growth. The discrete colonies could be obtained in the solid medium plates (Fig 3).

Table II. Biochemical test from solid media.

Source of the isolates	No. tested	NUMBER POSITIVE*										PRODUCTION OF		HYDROLYSIS OF			
		Gl	Fruc	Lac	Suc	Mal	Man	Sorb	Xyl	Sal	Dul	Catalase	Urease	Casien	Gelatin	Xanthine	Tyrosine
Biopsy (B)	12	12	12	1	10	12	7	3	1	9	0	12	10	12	12	0	0
Mouse foot pad Harvest (H)	12	12	12	0	09	06	9	2	1	7	3	12	08	12	12	0	0
Scraps/ Tissue fluid	12	12	12	0	07	07	6	0	1	9	0	12	09	12	12	0	0
	36	36	36	1	26	25	22	5	3	25	3	36	27	36	36	0	0

Gl=Glucose, Fruc=Fructose, Lac=Lactose, Suc=Sucrose, Mal=Maltose, Man=Mannitol, Sorb=Sorbitol, Xyl=Xylose, Sal=Salicin, Dul=Dulcitol. * Fermentation (acid only)

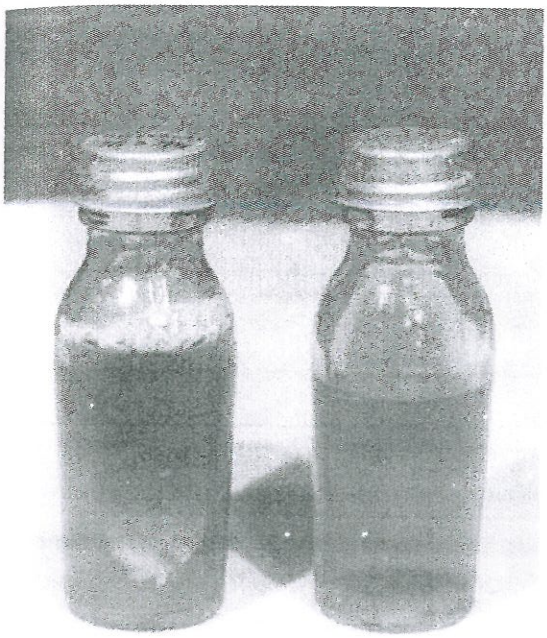

Fig. 2. Growth in biphasic medium showing a layer on the top and deposit in the medium.

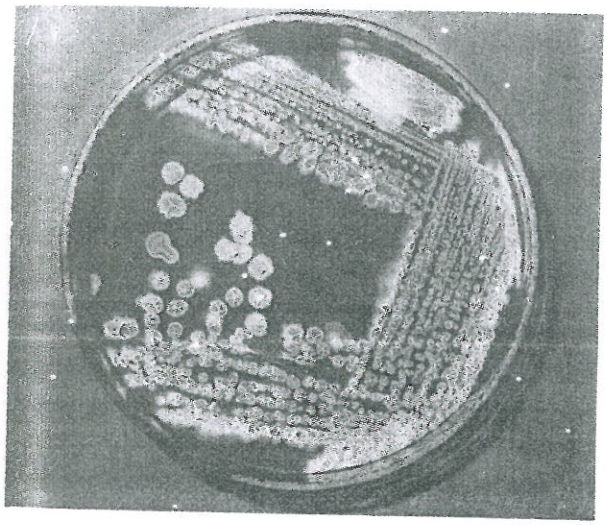

Fig. 3. Colony character on solid (CMY-PD agar) medium on 5th day of inoculation.

Incorporating drug in the medium, a total inhibition of growth was seen with Rifampicin and Oflaxicin upto concentration of 1.0 mcg per ml. Only 24 isolates were inhibited by DDS at a concentration of 100 mcg per ml. In disc diffusion all isolates inhibited by Oflaxicin, 16 were inhibited by DDS and 24 by Rifampicin in double diffusion technique (Fig 4)

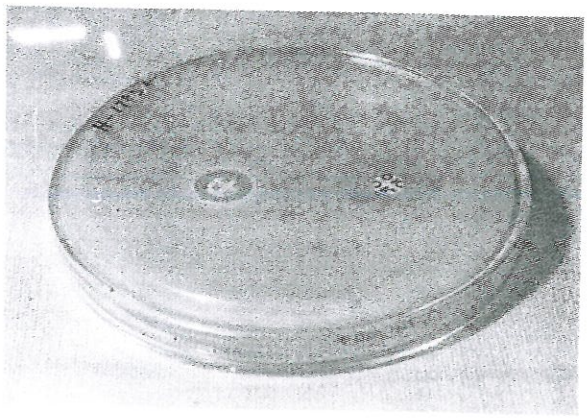

Fig. 4. Antimicrobial susceptibility tested by routine disc diffusion method.

Discussion

Isolations in the biphasic medium were successful in most of the cases. The growth could be observed by naked eye within 5 days of incubation. Although the organism in general resembled Dermatophilus congolensis, some differences have also been noted. The exact taxonomical placement of the organism therefore remains open till some more data are generated.

The growth in liquid part was characterized by a scum on the surface, a deposit at the bottom and a turbidity in the fluid portion. Such appearance of growth has also been noted previously in Hanks BSS and Earle's medium (Bhatia & Rao, 1989)[9] but intensity of growth was more in medium used in the present study. Moreover, a large number of isolates were studied this time and findings were consistent, uniform and reproducible.

In present study, the liquid medium without addition of CMY-PD agar showed less turbidity, less deposit and thinner surface layer compared to biphasic medium. The liquid medium with 0.5% CMY-PD agar showed growth similar to biphasic medium. This shows that adding of corn-meal-yeast-peptone

dextrose agar increases intensity of growth of leprosy organism in liquid medium. However which constituent of the medium is helpful must be worked out by further experimentation.

The serial subcultures from above isolates were possible in same medium and in solid media like CMY-PD agar and nutrient agar without change in the basic structures. The solid medium has advantage that the colonies can be recognized, studied and differentiated from those of other organisms including contaminants.

Incorporating drug in medium, a total inhibition of growth was seen with Rifampicin and Oflaxicin upto concentration of 1 mcg per ml. The inhibition was confirmed by subcultures on fresh medium. These findings are close to the clinical experience in leprosy and mouse foot pad results with M. leprae. Both Rifampicin and Oflaxicin are highly potent bactericidal drugs. Rifampicin is already in use in treatment of MB and PB leprosy as part of MDT regimens. The trials in different countries have shown Oflaxicin to be superior to conventional drugs. The low sensitivity of DDS should be understandable as the drug is much less effective than Oflaxicin and Rifampicin. Also the resistance to DSS (both secondary and primary) appeared long back and is highly prevalent in old and new cases.

It is interesting that using early growth, the antimicrobial susceptibility could be tested by disc diffusion method. If this is standardized the technique may be helpful in clinical leprosy practice. In our experience more number of isolates are found sensitive by disc diffusion technique and in low concentration of drug compared to drug-incorportation method. In natural course, the soft growth subsequently turns mycelial and sporulates heavily. Whether this affects dependability of technique in any way must be studied by test on larger number of isolates.

Table III. Effect of Antileprosy drugs on the isolates using drug-incorporation method.

Drug	No. of isolates tested	No. of isolates susceptible* in defferent concentration of drug (mcg/ml) 100	10	1.0	0.1
DDS	36	12	0	0	0
Oflaxicin	36	36	36	16	0
Rifampicin	36	36	36	24	0

*i.e. showed complete absence of growth in the concentration of drug used.

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