This can be done at the time of sampling if a sieve of appropriate mesh is inserted in the concentrating cylinder.

7. When stage I larvae are present, samples should be carefully sorted under a microscope.

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BACILLUS SPHAERICUS SPORE FROM SRI LANKA DEMONSTRATING RAPID LARVICIDAL ACTIVITY ON *CULEX QUINQUEFASCIATUS*

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ABSTRACT. A strain of *Bacillus sphaericus* isolated from coconut husk pits in Sri Lanka proved to be rapidly larvicidal to *Culex quinquefasciatus,* the local vector of Bancroftian filariasis at a dilution of 10^3 organisms per ml. A distinctive feature of this organism was that it contained a parasporal inclusion attached to the spore and was of a serotype hitherto unrecorded.

INTRODUCTION

Filariasis in Sri Lanka is endemic in the western and southwestern coastal belt of the island encompassing 1,200 km^2 and inhabited by about 2.5 million people. The human infection rate is about 1%.

The etiological agent of the disease is *Wuchereria bancrofti,* and the vector is a local strain of *Culex quinquefasciatus.* Mosquito breeding sites include inter-alia husk pits, discarded receptacles and catch pits. Husk pits are water filled excavations in the soil in which coconut husks are deposited and allowed to putrefy before the coir is prepared. The presence of numerous husk pits in this area renders them an important breeding site of *Cx. quinquefasciatus* (Lambrecht 1974).

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The efficacy of aerobic spore bearing bacteria, especially strains of *Bacillus thuringiensis* and *B. sphaericus*, in the biological control of insect vectors of disease is well established (Bulla et al. 1975). Recently, 2 new strains of aerobic spore bearing bacilli viz. *B. thuringiensis israelensis* (Goldberg and Margalit 1977, de Barjac 1978) and *B. sphaericus* 1593 (Ramoska et al. 1977) isolated from Israel and Indonesia respectively have proved to be rapidly entomicidal to a broad spectrum of mosquito larvae. This communication deals with the isolation of a similar entomopathogen effective against *Cx. quinquefasciatus* from husk pits in Sri Lanka.

MATERIALS AND METHODS

ISOLATION OF AEROBIC SPORE BEARING BACILLI. One liter of water was collected from a pit devoid of mosquito larvae in an area of husk pits yielding a high larval density situated about 25 mi. from Colombo. The water was centrifuged and the deposit kept at 80°C for 10 min to destroy vegetative bacteria. A loopful was streaked on 5% blood agar and incubated aerobically at 36° C for 24 hr to obtain discrete colonies. The balance of the deposit was enriched in nutrient broth and treated as above. Each colony with distinctive characteristics was subcultured on egg slopes and preserved at −4°C. Nine such isolates were made in all. Identification was not attempted at this stage.

PREPARATION OF INSECT TRAYS. Larvae for the studies were hatched from eggs obtained from field collected females of *Cx. quinquefasciatus*.

Glass dishes (19.5 x 13 x 8.0 cm) were set up each filled with 1000 ml of tap water, and 100 first instar larvae were introduced into each dish with a larval pipette. These were fed with finely-powdered sieved toasted brown bread at daily intervals. The larvae were maintained in an insectary where the maximum and minimum temperature was 32.2°C and 26.6°C respectively and relative humidity about 80%.

PREPARATION OF INOCULUM. Each culture was emulsified in nutrient broth, streaked on 5% blood agar and incubated at 37°C for 48 hr. If pure, colonies were picked into 1 liter flasks of nutrient broth and incubated at 37°C for 48 hr. The broth cultures were centrifuged after a purity check and re-suspended in 10 ml of N. saline. Serial dilutions ranging from 10^{-1} to 10^{-7} were made from 1 ml of the suspension and viable counts performed employing the Miles and Misra (1938) technique. The deposit was diluted so as to yield bacterial concentrations ranging from $10^{1} - 10^{6}$ or more per ml of water on inoculation into the experimental trays.

MEDIA EMPLOYED. The medium employed for growing the culture was nutrient broth. The formula—Lab Lemco 10 g, peptone 10 g, NaCl 5 g, water to 1 liter. This medium was gelled with 2% agar and 5% human blood added to make up the blood agar.

ENTOMOPATHOGENIC CHALLENGE. Three larval trays were employed for each dilution of the inoculum. Appropriate dilutions of the initial deposits were made so as to yield bacterial counts ranging from 10^{1}—10^{8} org/ml in the inoculation trays. Each tray was gently stirred with a separate glass rod. Identical mosquito trays inoculated with similar volumes of N. saline served as controls. Counts of dead/living larvae and pupae were made at 24 hr up to the end of pupation. The experiments were repeated thrice.

RESULTS AND DISCUSSION

Nine aerobic spore-bearing bacilli in all were tested in this manner. One of them designated MR 4 showed marked larvicidal activity. The lowest dosage ($1X10^{3}$ org/ml) of this strain killed almost all exposed larvae as did the 5 dosages up to $1X10^{5}$ org/ml.

This organism grew readily on blood and nutrient agar yielding haemolytic colonies 2–3 cm in diameter with entire edge and rough surface (Figure 1). Gram stained films showed weakly Gram posi-

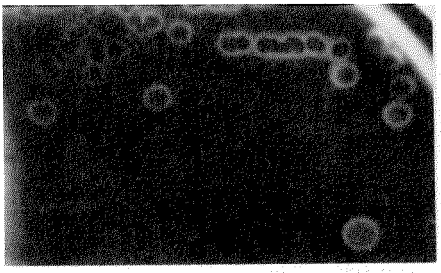

Fig. 1. *Bacillus sphaericus* MR 4 48 hours on blood agar, X 6 Grey colonies with surrounding β haemolysis.

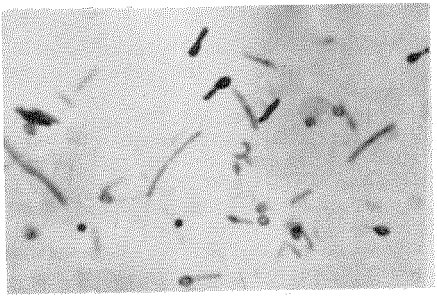

Fig. 2. *Bacillus sphaericus*. MR 4 X 1800. Weakly gram positive bacilli. round terminal and free spores.

tive 2–6μmX 1μ m bacilli with round terminal spores (Figure 2). It was provisionally identified by its physiological and biochemical characteristics as a strain of *Bacillus sphaericus* (Cowan 1974) and subsequently confirmed by de Barjac (1979, personal communication) at the Pasteur Institute, Paris. Unusual features of the bacillus were that it contained a parasporal body (thus resembling *B. thuringiensis*) contiguous with the spore and was of a serotype unrecorded previously.

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