ISOLATION AND IDENTIFICATION OF A *BACILLUS* SPECIES PATHOGENIC TO MOSQUITO LARVAE: ITS COMMERCIAL IMPLICATIONS AS BIOINSECTICIDE

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ABSTRACT

An aerobic spore former possessing larvicidal activity against mosquitoes was isolated from sludge samples. Following various biochemical and morphological tests using standard procedures, the organism was identified as a strain of *Bacillus sphaericus*. Toxicity against mosquito larvae appeared well before the synthesis of dipicolinic acid and the development of heat stable forms. The commercial significance of this bacterium as a possible bioinsecticide is suggested.

INTRODUCTION

RESURGENCE of mosquito-borne diseases in India is growing at an alarming rate because mosquito control programmes using chemical insecticides have not been successful. Moreover, these insecticides cannot be used continuosly with impunity because of a variety of reasons such as their adverse impact on environment and the development of vector resistance in mosquitoes[1–3]. This alarming situation prompted us to develop an alternative method such as bioinsecticides[4,5], as they are selective towards their targets[6], biodegradable[7] and more importantly avoid a rebound of vector resistance occurring frequently after chemical insecticide treatments[8–10].

An organism possessing larvicidal activity against mosquitoes was isolated and the levels of its pathogenicity with various available bioinsecticides were compared.

MATERIALS AND METHODS

Isolation and Identification: Extracts were prepared by mixing 1 g sludge sample in sterilized distilled water (10 ml) and filtered. The filtrate was diluted and appropriate dilutions were heated at 70°C for 15 min before inoculating a liquid medium. The flask was incubated on a rotary shaker at 30 ± 1°C. Spores formed after 30 hr of incubation were diluted and suitable dilutions plated on nutrient agar in petri plates after heat treatment at 70°C. The colonies developed in the petri plates after 30 hr of incubation were grown individually in liquid medium for 24 hr followed by centrifugation at 10,000 g for 10 min and the pellet was used for toxicity bioassay (procedure given below) against second instar larvae of *Culex pipiens* mosquitoes. The colony which gave the maximum toxicity was chosen for further investigations. For identification, various biochemical and morphological tests such as fermentation of sugars, citrate utilization etc were followed adopting standard procedures[11].

Cultural conditions: The organism was grown in a liquid medium containing (g/litre of distilled water):– $FeSO_4.7H_2O$, 0.01; $MnSO_4$, 0.1; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.08; K_2HPO4, 0.025; yeast extract, 2; peptone, 4; D–glucose, 1 and casein, 5. Solutions of yeast extract, peptone, casein, D–glucose, K_2HPO_4 and $CaCl_2$ were separately prepared, sterilized and added before inoculation. The pH of the medium was adjusted to 7.1 before sterilization.

Measurement of growth: The organism was grown in a liquid medium by following an active culture procedure[12]. Growth was monitored by measuring optical density at 600 nm with a spectrophotometer (Carl Zeiss). The protein, heat stable forms (HSF) and dipicolinic acid (DPA) were determined by following the normal procedures[13–15].

Bioassay of larvicidal activity: At different time intervals, samples were withdrawn, suitably diluted and used for larvicidal activity. Bioassays were performed on second instar larvae of *Culex pipiens* suspended in 10 ml of deionized water. Routinely, three petri plates containing 50 larvae were tested for every 10-fold dilutions with 2 petri plates each containing 50 larvae serving as controls. Larval death was recorded at 12 hr intervals for 36 hr. The

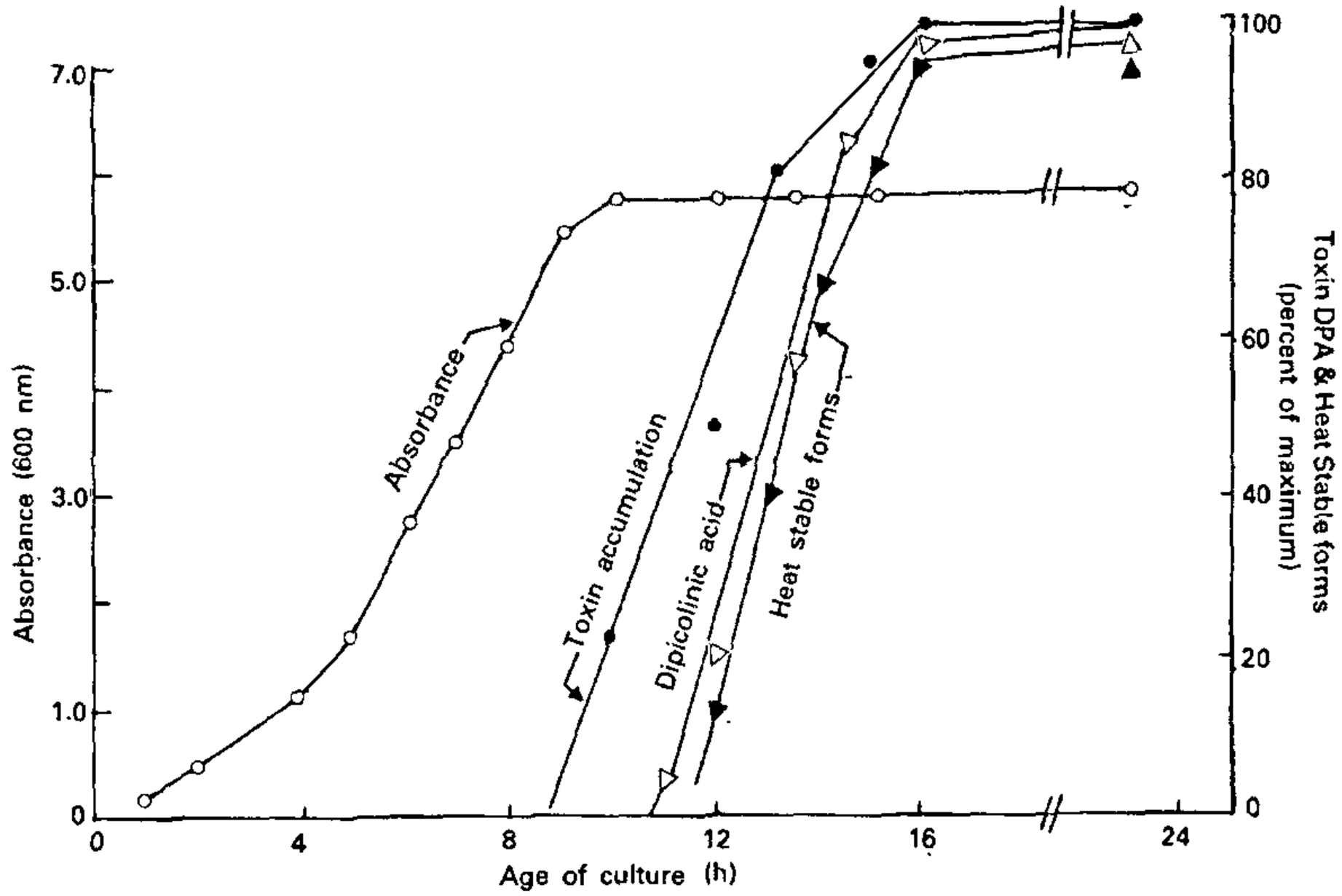

Figure 1. Appearance of toxin, dipicolinic acid and heat stable forms in a laboratory isolated strain of *B. sphaericus*.

toxic activity is represented as LD_{50} value which is defined as the amount of protein present in the cells needed to kill 50% larvae in 36 hr

RESULTS AND DISCUSSION

An aerobic spore former isolated from sludge sample collected from a stagnant pond was gram-positive, ferment D-glucose, D-mannose and D-fructose but failed to hydrolyse starch and gelatin. The organism forms smooth opaque colonies on nutrient agar, the vegetative cells were rod-shaped (average size 3.2×0.9 μm), did not form chains and were highly motile during the logarithmic growth phase. The spore was round (average size 0.61 μm) with bulged sporangium. On the basis of various biochemical and morphological tests and following

Table 1 *Comparison of larvicidal activity with various bioinsecticides and strains of Bacillus sphaericus*

Organism/Strain	Bioinsecticide (Trade name)	Source	LD_{50} value (ng/ml)
B. thuringiensis Var. *israenlensis* (commercial preparation)	IPS-78	WHO	17.2
	IPS-80	WHO	13.5
	Vectobacter	Abbott's lab	20.0
Pure spores of *B. thuringiensis* Var. *israelensis*		Dr Deberjac (France)	15.0
Bacillus sphaericus strains:-			
SS-II-1		Dr Davidson (USA)	100.0
1404		"	450
1881		"	500.0
1553		Dr Yousten (USA)	19.0
B. sphaericus (RRLJ)		Jorhat	5–7

Toxicity assays were done as mentioned in the text.

the Bergey's Manual of Determinative Bacteriology the organism was identified as a strain of *B. sphaericus*. This was confirmed by Dr Ruth E. Gordon of New Jersey.

The toxin appeared during stationary growth phase and accumulated intracellularly in the sporulating cells before DPA synthesis and appearance of HSF (figure 1). These parameters were used as markers and seem to have no direct correlation with toxin synthesis. This was confirmed with the isolation of asporogenous mutant of this organism synthesizing toxin comparable to parent strain. Interestingly no toxin was synthesized during logarithmic growth phase in both mutant and parent organism. The toxin is proteinous in nature, partially purified from sporulating cells and the toxic activity was destroyed by treatment with proteolytic enzymes. The dry spore powder preparation possessing larvicidal activity was prepared which can withstand storage for more than 10 months at room temperature without loss either in spore viability or toxicity. This information is quite interesting as the organism may be exploited for making bioinsecticide against mosquitoes. The data presented in table 1 clearly showed that the toxin level in this bacterium is quite high or atleast comparable to the best commercially produced bioinsecticides or even many strains of *B. sphaericus* and *Bacillus thuringiensis* available from various sources. These data further suggest that this organism may be used as bioinsecticide against mosquitoes.

10 November 1986; Revised 28 January 1987.

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NEWS

MORE WOMEN DYING FROM LUNG CANCER

Lung cancer is gradually replacing breast cancer as the main cause of cancer deaths among women in industrialized countries.

WHO has recently analysed female lung cancer mortality in 28 developed countries and compared the ratios of age-standardized death rates from breast cancer and from lung cancer in 12 countries reporting the highest figures over the past two decades. Death rates (adjusted for age) increased by 200% in Australia, Ireland, New Zealand, and the United Kingdom and by 300% in Canada, Denmark, and the USA. This trend may well reflect an earlier breakdown of social taboos against female smoking in industrialized countries, which is particularly apparent in English-speaking countries.

Lung cancer is a self-induced, avoidable and preventable tumour, and these WHO findings clearly indicate the need to establish new priorities and strategies in cancer control. (*World Health Forum*, 1987, Vol. 8, No. 1, p. 116, World Health Organization, 1211 Geneva, Switzerland).