STUDIES ON "SAPPE" DISEASE OF THE SILKWORM *BOMBYX MORI*, L.

I. Isolation and Characterization of Pathogenic Bacteria from Diseased Worms

C. CHITRA, ARUNA BHANDARKAR, N. G. K. KARANTH AND V. N. VASANTHARAJAN
Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore-560012

ABSTRACT

"Sappe" disease of the silkworm causes great economic loss in Mysore State. Several species of bacteria, namely, *Aerobacter cloacae, Achromobacter superficialis. Achromobacter delmarvae, Pseudomonas boreopolis, Pseudomonas ovalis, Escherichia freundii,* and *Staphylococcus albus* were isolated from various tissues of diseased silkworms. They were proved to be pathogenic by artificial introduction through oral feeding causing a mortality of 37·5 to 65% and a reduction of 27 to 43% in the cocoon weight of the surviving larvae. The symptoms of the disease are described. Based on the above results, it is concluded that "Sappe" includes a class of diseases exhibiting similar symptoms rather than a single malady.

INTRODUCTION

EIGHTY per cent of the silk produced in India comes from Mysore State, where sericulture is the main livelihood of a substantial part of the population. The silkworms are affected by several diseases caused by micro-organisms[1-2]. In Mysore State, a type of flacherie, popularly called "Sappe" by the farmers, frequently causes heavy crop loss during the monsoon season especially after a dry spell. The disease is rather ill-defined and is commonly noticed in the final instar; the general symptoms observed are sluggishness, loss of appetite and slow softening of the body. There is about 20 to 40% mortality and the surviving larvae spin poor cocoons. Though the disease has been prevalent for a long time, very little is known about the causative organism(s) and much less about its prevention and control. This paper reports the isolation and characterization of several strains of bacteria from diseased silkworms. The pathogenicity was also established by artificial introduction through oral feeding.

MATERIALS AND METHODS

Collection of silkworms.—Diseased silkworms were collected from endemic areas of Channapatna Taluk in Mysore State. The normal cross-bred silkworms (*Bombyx mori,* L.; *Mysore* × *C. nichi*) obtained from the grainage (the authorized rearing centres which supply layings for the farmers) were maintained in the laboratory.

Isolation, purification and characterization of the organisms from diseased silkworms.—The organisms were isolated from silkworm haemolymph, mid-gut and silk glands. Anaesthetized worms were surface-sterilized by wiping with 70% ethanol and dissected under aseptic conditions. One loopful of haemolymph was directly transferred onto a nutrient agar slant. Tissues such as intestines and silk glands were cut into small pieces and suspended separately in physiological saline. One loopful of each suspension was then inoculated onto nutrient agar slants which were incubated at 30° C. The bacteria that developed on each slant were purified by routine methods[3] and identified by following Skerman's key[4] and Bergey's manual[5].

Pathogenicity of the isolates.—Among the bacterial isolates obtained, seven strains were chosen for detailed virulence studies. Cell suspensions in distilled water (10^6 bacteria/ml) were smeared on mulberry leaves and fed to V instar larvae (10,000 cells/worm). The worms were provided with inoculated leaves at the following stages: (*i*) immediately after they came out of IV moult, (*ii*) twice on the second day of V instar, and (*iii*) once on the third day. Forty worms were kept under each treatment. For control, the worms were given mulberry leaves smeared with the same quantity of water. The weight of the worms was recorded from 4th day onwards till the spinning stage. Symptoms of disease noticed in them were also recorded every day. When the disease manifested, a sample worm from each batch was dissected out and the pathogen was looked for in various tissues following the isolation methodology detailed above. After emergence of the moths, the cocoons were weighed and the number of moths which emerged was also counted.

RESULTS AND DISCUSSION

Intestinal flora of healthy worms.—The intestine of the healthy silkworm is generally reported to contain very few microorganisms both in varieties and numbers[6-8]. Our preliminary observations were also similar; detailed reports on the microflora of the healthy silkworms will be reported elsewhere.

Types and distribution of bacteria in diseased silkworms.—Representative colonies and those with dissimilar appearance that developed on the plates were picked, purified and identified in the usual

way. The types of bacteria and the number of instances they were encountered in the different organs are presented in Table I. While the silk

TABLE I

Bacteria isolated from diseased silkworms

Tissue	Bacterial isolate	Frequency*
Haemolymph	*Aerobacter cloacae*	3/6
	Achromobacter delmarvae	2/6
	Achromobacter superficialis	1/6
Mid-gut	*Aerobacter cloacae*	6/6
	Achromobacter superficialis	3/6
	Achromobacter delmarvae	2/6
	Pseudomonas boreopolis	2/6
	Pseudomonas ovalis	1/6
	Escherichia freundii	1/6
	Staphylococcus albus	1/6

* Number of instances the organism was obtained out of 6 diseased worms tested.

glands were found to be sterile, organisms could be isolated from haemolymph in three out of six cases. The intestine contained a much greater variety of organisms which could be recovered at varying frequencies. The fact that *Aerobacter cloacae* was encountered in all the diseased worms tested, strongly suggests that it could be a potential pathogen. *Achromobacter superficialis* was the next most commonly isolated organism. It is of interest to note that both these organisms have been implicated in causing diseases of silkworms by Japanese workers[9]. Except for *Pseudomonas ovalis* the other organisms are reported for the first time from the intestines of diseased silkworms.

Pathogenicity trials.—All the isolates when fed to the worms caused the disease with varying severity and with different rates of mortality (Tables II and III). *Aerobacter cloacae* and *Achromobacter superficialis* have also been found by Japanese workers to be pathogenic. Interestingly they have also reported that *Pseudomonas ovalis* isolated from diseased silkworms did not cause any damage on re-infection[9]. On the other hand, we have observed that introduction of *Pseudomonas ovalis* results in 65% mortality. This could be due to differences in (*i*) race of the silkworm used, (*ii*) strain of *Pseudomonas ovalis*, (*iii*) inoculum load, etc. The pathogenicity of other organisms, *viz.*, *Achromobacter delmarvae*, *Pseudomonas boreopolis*, *Staphylococcus albus* and *Escherichia freundii* are being reported for the first time. However, we could not obtain in our studies *Bacillus cereus* var. *alesti*[10], *Streptococcus faecalis—Streptococcus faecium intermediate* E-5[11], *Serratia piscatorum*, *Serratia marcescens*, *Proteus vulgaris*, *Proteus inconstans*, *Proteus morganii*, *Aerobacter aerogenes*[12], *Micrococcus flavus* and *Micrococcus candidus*[13] isolated from diseased worms and reported to be pathogenic by others. It should also be mentioned here that the pathogenicity trials were carried out by us on normal larvae fed with ordinary mulberry leaves from the garden unlike Japanese workers, who worked with axenically reared larvae fed with synthetic diets.

TABLE II

Mortality rate in silkworm due to infection with bacterial isolates from diseased larvae

Bacteria	% mortality			
	Age in days (V instar)*			
	5	6	7	8
Control (no bacteria)	0·0	0·0	0·0	5·0
Aerobacter cloacae	0·0	0·0	2·5	65·0
Achromobacter superficialis	0·0	5·0	7·5	37·5
Achromobacter delmarvae	0·0	2·5	10·0	65·0
Staphylococcus albus	0·0	0·0	0·0	62·5
Escherichia freundii	0·0	12·5	12·5	62·5
Pseudomonas ovalis	0·0	0·0	5·0	65·0
Pseudomonas boreopolis	2·5	5·0	5.0	65·0

* There was no mortality in any instance upto 5 days.

The rate of mortality in the treated silkworms is given in Table II. Table III gives the data on the effect of feeding the worms with bacterial isolates on growth, cocoon weight and emergence. It was seen that these cause a remarkable loss of weight of larvae as well as cocoons (Fig. 1 and Table III). As a further confirmation of pathogenicity we could re-isolate the inoculated organism in every instance.

Symptoms of the disease.—There is no scientific description of the disease "Sappe" available in the literature. "Sappe" in Kannada means "sluggish", referring to the sluggish habits of the infected larvae. In our experiments, the worms did not show any external manifestation of "Sappe" until the disease was far advanced. The symptoms appeared on the 4th or 5th day of V instar in all the three batches used. The infected worms had poor appetite, generally refused to feed and regurgitated a clear brownish fluid. Mature leaves were not eaten at all by the infected larvae; however, they consumed very tender leaves with reluctance. The body finally became very soft, the head being swollen in some cases. The integument was wrinkled and writhing movements were noticed. Unequal development of worms in the same batch was also very striking.

It is interesting to note that almost similar symptoms are caused by a large variety of microorganisms. Very little is known about the pathology and progress of the disease in silkworm due to

invasion of these bacteria and these aspects are being actively investigated in this laboratory. It is also known that a sudden shift in the quality and quantity of intestinal microflora due to physiological changes can result in pathological conditions. Organisms that are normally harmless may turn out to be harmful if their population increases, or if they form a new product in an altered environment[14,15]. "Sappe" frequently breaks out in the rainy season. We do not as yet know whether our isolates act in the above ways or are potential pathogens by themselves. The source of infection,

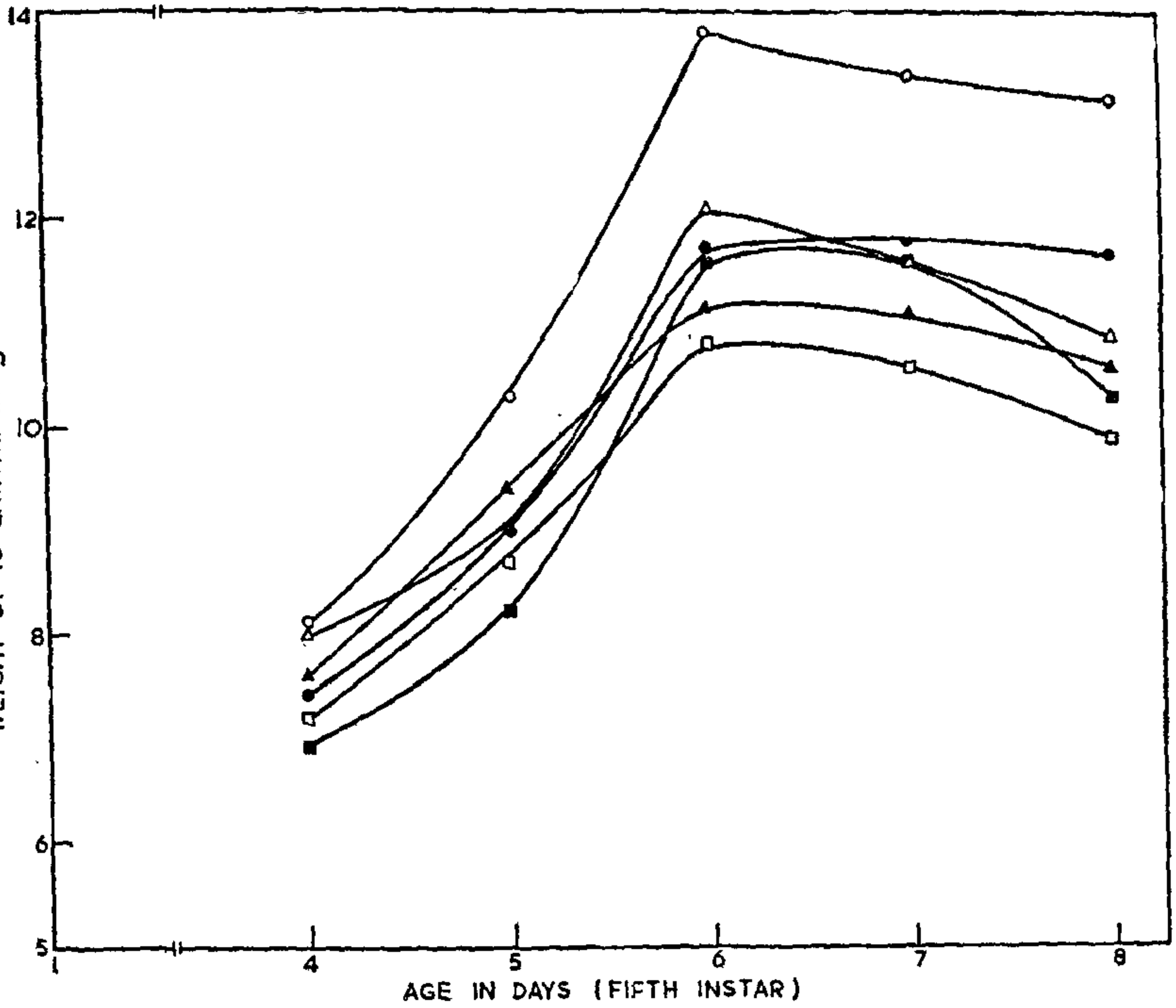

FIG. 1. ○—○ Control. △—△ infected with *Aerobacter cloacae*. ▲—▲ infected with *Achromobacter delmarvae*. ■—■ infected with *Staphylococcus albus*. ●—● infected with *Escherichia freundii*. □—□ infected with *Pseudomonas ovalis*

TABLE III

Effect of infecting with bacterial isolates on the growth and cocoon weight of silkworm larvae

Bacterial isolate used	Wt. of 10 larvae on 6th day of V instar (g)	% reduction in wt.	% mortality on 8th day of V instar	% emergence of moths†	Wt. of one cocoon in mg*	% reduction in cocoon weight
Control (no organisms)	13·8	..	5.0	86·8	96·9	
Aerobacter cloacae	12·1	12·3	65·0	57·1	64·8	33·1
Achromobacter superficialis	11·1	19·6	37·5	92·0	64·0	34·0
Achromobacter delmarvae	10·0	27·5	65·0	100·0	58·5	39·7
Staphylococcus albus	11·6	15·9	62·5	83·4	70·4	27·4
Escherichia freundii	12·4	10·1	62·5	75·8	55·3	43·0
Pseudomonas ovalis	10·8	21·7	65·0	64·3	67·7	30·4
Pseudomonas boreopolis	11·0	20·3	65·0	100·0	57·6	40·6

* Significant at 5% level. Minimum difference for significance 5·5 mg, mcm, of 5-10 cocoons.
† % of moths emerged out of number of larvae pupated.

mode of spread and probable preventive measures will be investigated. Of course it should be stressed that "Sappe" is a farmer's term and perhaps be applied to a class of disease having similar symptoms rather than to denote any single ailment.

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GROWTH DYNAMICS AND DEVELOPMENTAL PATTERNS IN THE UNICELLULAR TRICHOMES OF ANGIOSPERMS

N. RAMAYYA AND M. PRABHAKAR

Plant Anatomy and Taxonomy Laboratory, Department of Botany, Osmania University, Hyderabad-500007, India

ABSTRACT

Growth dynamics and developmental patterns of unicellular trichomes of angiosperms based upon calibration of specific growth components at critical stages of their ontogeny is presented. The stigmatic papillae of *Chrysanthemum carinatum* L., stigmatic hairs of sunflower (*Helianthus annuus* L.) and filament hairs of *Lagascea mollis* Cav. have been studied for the purpose. The trichomes bear a cuticular membrane right from the onset of ontogeny and their entire outer wall participates in differentiation rather than a predefined locus as in root hairs. Nuclear movement is independent of the elongation of the cytoplast and of the cell wall, but markedly slows down in the last phase of trichome development, thus exercising its control on trichome growth, while being farther behind the trichome tip unlike in root hairs. The nucleus in its interphase is highly plastic, expanding or contracting according to the available space, but without detriment to its function. No inhibitory field effects of Bunning operate during the ontogeny of the trichomes, since they not only develop abutting over one another, but also simultaneously. As a part of cellular adjustment during growth, the lateral walls of trichomes which in their proximal region are organically connate with those of adjacent trichomes, show separation at their outward margin in *Lagascea mollis*. Depending on the trichome concerned, either the ontogeny involves both symplastic and apical intrusive growth or only the former. During development, trichome diameter and length show continuous increase; the nucleus also similarly shows increase, in its diameter. Four developmental patterns are so far recognisable in the unicellular trichomes of angiosperms including that of the root hairs.

INTRODUCTION

AMONG the unicellular trichomes of angiosperms, the root hairs[1,4,5] and cotton hairs[6,7] are the most extensively studied about growth and differentiation. But these investigations, however, did not identify specific growth components and study them at appropriate ontogenetic stages, so that there is presently no precise information on the growth changes or dynamics of these trichomes. Understanding of the latter is valuable not only in relation to the trichomes themselves, but also regarding the general cellular growth patterns which is not feasible to follow in the usual plant tissues