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The Disease Complex of the Gypsy Moth

II. Aerobic Bacterial Pathogens

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Eighty-six pathogenic aerobic bacterial isolates from diseased gypsy moth larvae collected in both sparse and dense populations were characterized and identified as members of the families Bacillaceae, Enterobacteriaceae, Lactobacillaceae, Pseudomonadaceae, and Achromobacteraceae. The commonest pathogens were *Streptococcus faecalis, Bacillus cereus, Bacillus thuringiensis,* Group C *Enterobacter* types, and *Pseudomonas* spp. However, *S. faecalis* and *B. cereus* were found operating only in the dense populations. The wide variety of potentially pathogenic bacteria encountered may indicate that, under conditions of high host density, these bacteria may contribute significantly to the population dynamics of the gypsy moth.

INTRODUCTION

A previous study (Campbell and Podgwaite, 1971) undertaken to elucidate the impact of disease on natural populations of the gypsy moth, *Porthetria dispar,* revealed that certain bacterial isolates from diseased larvae were pathogenic to this insect. Sixty-three of these isolates were associated with larvae taken from dense populations while 23 were associated with sparse-population larvae.

Recent reports (Cosenza and Lewis, 1965, 1966; Doane, 1970; Doane and Redys, 1970) have named only variants of *Streptococcus faecalis* and *Bacillus thuringiensis* as primary bacterial pathogens of this insect. There have been no reports of surveys conducted to determine what other bacterial pathogens operate in natural populations of this insect and/or how they may contribute to its population dynamics. Studies were undertaken to identify the pathogenic isolates and to determine the differences existing between the two population types in this regard.

METHODS

The methods for collecting diseased larvae and the subsequent isolation and pathogenicity testing of bacteria have been described (Campbell and Podgwaite, 1971).

Identification of Bacterial Pathogens

Colonial, morphological, and physiological characteristics of the pathogenic bacteria were determined, using standard bacteriological techniques according to "The Manual of Microbiological Methods" (Society of American Bacteriologists, 1957) and the keys of Breed et al. (1957) and Skerman (1967) unless otherwise indicated.

All isolates were incubated for 18 hr on trypticase soy agar slants (BBL) at 30°C, gram stained, and grouped to family according to the scheme outlined in Fig. 1.

Motility and cellular arrangement were determined from wet mounts of 4- to 12-hr trypticase soy broth (BBL) cultures. Motility of streptococcal isolates was determined in streptococcus antigen broth, which consisted of the following ingredients in grams

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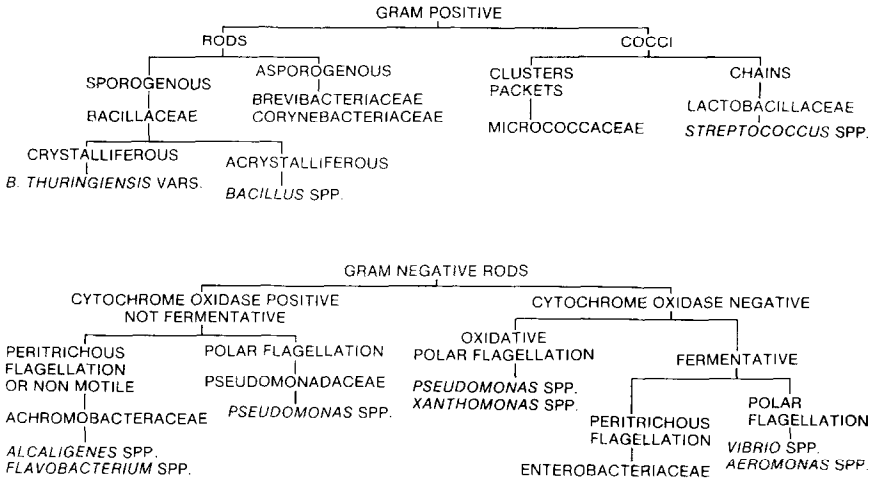

FIG. 1. General scheme for identifying aerobic bacterial pathogens.

per liter: Bacto beef heart, 45; proteose peptone, 20; glucose, 4; K_2HPO_4, 10; *p*H 7.4. Flagellar arrangement was detected according to Leifson (1951). Endospore stains were made by staining cells with 5% malachite green and counterstaining with 1% aqueous safranin. Crystalliferous bacilli were determined by staining smears from sporulating cultures by a modification of Smirnoff's (1962) technique, using 2% aqueous safranin as the counterstain.

The anaerobic fermentation of glucose was determined in the medium of Hugh and Leifson (1953). Oxidase activity was detected with Kovac's (1956) method.

Bacilli were identified by following the keys of Wolf and Barker (1968) and streptococci according to Sharpe et al. (1966). For serological typing, polysaccharide extracts of streptococci were prepared from 24-hr streptococcus antigen broth cultures by the method of Rantz and Randall (1955). The precipitin test was performed in capillary tubes, utilizing group D antisera obtained from BBL. Members of the Enterobacteriaceae were identified by the use of methods described by Edwards and Ewing (1962). No attempt was made to identify species within the Pseudomonadaceae or the Achromobacteraceae.

RESULTS

The bacterial pathogens were found to represent the families Bacillaceae, Lactobacillaceae, Enterobacteriaceae, Pseudomonadaceae, and Achromobacteraceae. Four isolates of short, gram-positive, asporogenous rods were unfortunately lost upon subculture and could not be identified positively.

The characteristics of 15 isolates belonging to the family Bacillaceae are shown in Table 1. Eight of these were identified as *Bacillus cereus* and 7 as varieties of *Bacillus thuringiensis*.

Table 2 lists the characteristics of 26 streptococcal isolates. These were the only members of the Lactobacillaceae encountered that were pathogenic, and all were identified as varieties of *Streptococcus faecalis*. All were α-hemolytic, grew at 10°C, 45°C, and *p*H 9.6, but not at 50°C, survived at 60°C for 30 min, reduced tellurite, fermented glycerol anaerobically, and were serologically typed as Lancefield Group D.

Twenty pathogenic isolates were identified as members of the family Enterobacteriaceae. Their biochemical characteristics are shown in Table 3. The majority of these were Group C *Enterobacter* types; nonpigmented strains of *Serratia marcescens* and a

Proteus sp. were also represented. Two members of the group were not identified.

The remaining pathogens were all nonfermentative, gram-negative rods (Table 4). The majority of these were identified as *Pseudomonas* spp.; the genus *Alcaligenes* was represented by two isolates.

The distribution of bacterial pathogens among sparse and dense population sites is shown in Table 5. All but one of the *Bacillus* isolates and all varieties of *S. faecalis* were found operating only in the dense populations. About twice as many pathogenic pseudomonads were isolated from the sparse population as from the dense, whereas isolates of the Enterobacteriaceae were about equally divided between sparse and dense sites.

Discussion

Previous studies (Cosenza and Lewis, 1965; Doane 1970) have indicated that certain varieties of *Streptococcus faecalis* are pathogenic in natural gypsy moth populations and may be responsible for epizootics. Results of our survey confirm the involvement of this microorganism in the disease complex of this insect—but only in dense populations, and not in epizootic proportions.

The fact that this bacterium was never isolated from sparse-population larvae is surprising, because it is an enteric microorganism; and other enterics such as Group C *Enterobacter* spp. were found operating in these populations. The absence of *S. faecalis* may reflect the absence of a particular mammalian or avian vector of this bacterium in these populations, or perhaps the microorganism is present in these populations; but because of low host densities or

TABLE 1

Characteristics of Gram-Positive, Catalase-Positive, Aerobically Sporulating Rods

Characteristic	Reaction	
Oval spores	+	+
Swollen sporangia	−	−
Vacuolation	+	+
Parasporal inclusions	−	+
Growth at 60°C	−	−
Growth at pH 6.0	−	−
Anaerobic growth in glucose broth	+	+
Acid from mannitol	d	−
Lecithinase	+	+
Number of isolates	8	7
Identification	*Bacillus cereus* vars.	*Bacillus thuringiensis* vars.

+ = positive reaction or characteristic; − = negative reaction or characteristic; d = different biochemical types.

TABLE 2

Characteristics of 26 *Streptococcus faecalis* Isolates

Test	Reaction[a]
Lancefield group D	+
Hemolysis	α
Growth at 10°C	+
Growth at 45°C	+
Growth at 50°C	d
Survive 60°C/30 min	+
Growth at *p*H 9.6	+
Tellurite reduction	+
Glycerol (anaerobic)	+
Litmus milk	Reduced, acid
Gelatin	−
Pigmentation	Yellow, water-insoluble
Motility	d
Starch	−
Glucose	+
Lactose	d
Trehalose	+
Sucrose	+
Maltose	+
Mannitol	+
Arabinose	d
Meletzitose	d
Melibiose	d
Salicin	+

[a] + = positive reaction or characteristic; − = negative reaction or characteristic; d = different biochemical types.

TABLE 3

CHARACTERISTICS OF THE OXIDASE-NEGATIVE, NITRATE REDUCING, FERMENTATIVE, GRAM-NEGATIVE RODS

Characteristic	Reaction[a]					
Indole	−	−	−	+	−	−
Methyl red	+	d	+	+	+	+
Voges-Proskauer	d	+	+	−	−	−
Citrate	+	+	+	+	+	+
H_2S	−	−	−	−	−	−
Urease	−	−	−	+	−	−
KCN	+	+	+	+	+	+
Motility	+	+	+	−	−	−
Gelatin	+	+	−	−	−	−
Lysine decarboxylase	+	+	+	−	−	−
Arginine dihydrolase	−	−	−	−	−	−
Ornithine decarboxylase	+	+	+	−	−	−
Phenylalanine deaminase	−	−	−	+	−	−
Gas from glucose	+	−	+	−	−	−
Lactose	d	−	−	−	+	−
Sucrose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Dulcitol	−	−	−	−	−	−
Salicin	+	+	+	−	+	+
Adonitol	d	+	−	−	−	−
Inositol	d	+	−	−	−	−
Sorbitol	+	+	−	−	+	−
Arabinose	+	−	+	−	−	−
Raffinose	d	−	−	−	−	−
Rhamnose	d	−	+	−	−	−
Number of isolates	12	3	1	1	1	1
Identification	Group C *Entero-bacter* spp.	*Serratia marcescens*	*Hafnia* sp.	*Proteus* sp.	Unidentified	

[a] + = positive reaction or characteristics; − = negative reaction or characteristic; d = different biochemical types.

TABLE 4

CHARACTERISTICS OF NONFERMENTATIVE, GRAM-NEGATIVE RODS

Characteristic	Reaction[a]					
Oxidative in Hugh's medium	+	+	−	−	+	−
Cytochrome oxidase	+	−	+	+	−	−
Motility	+	+	+	+	−	−
Polar flagellation	+	+	+	+	−	−
Fluorescent pigment	+	+	+	−	−	−
Number of isolates	10	3	2	2	1	2
Identification	*Pseudomonas* spp.					*Alcaligenes* spp.

[a] + = positive reaction or characteristic; − = negative reaction or characteristic.

TABLE 5

DISTRIBUTION OF BACTERIAL PATHOGENS AMONG SITES FROM SPARSE AND DENSE POPULATIONS AND PERCENT MORTALITY CAUSED IN FEEDING TRIALS

Pathogen	Number of isolates							$\bar{x}$ % mortality produced in feeding trials
	Dense sites[a]			Sparse sites[b]				
	1	2	3	1	4A	5	9A	
Bacillus cereus	4	3	1	—	—	—	—	49
Bacillus thuringiensis	3	—	3	1	—	—	—	46
Streptococcus faecalis	14	10	2	—	—	—	—	71
Enterobacter Group C	5	2	1	—	3	1	—	30
Hafnia sp.	—	—	1	—	—	—	—	10
Serratia marcescens	1	1	—	—	—	—	1	60
Proteus sp.	—	—	—	—	1	—	—	10
Unidentified Enterobacteriaceae	—	—	—	1	1	—	—	10
Pseudomonas spp.	1	4	1	1	9	—	4	27
Alcaligenes spp.	1	1		—	—	—	—	15
Unidentified	2	1	1		—	—	—	20
Total	31	22	10	3	14	1	5	—
Total insects examined	388	321	248	22	167	38	39	—

[a] >4,000 egg masses per acre.
[b] <25 egg masses per acre.

undetermined environmental factors, is not likely to come into contact with the host insect. The virtual absence of pathogenic *Bacillus* spp. in sparse population larvae could be rationalized on the basis of low dispersal capacity of these sporeformers, coupled with low insect densities.

Although a variety of bacteria were isolated and found pathogenic for early-instar larvae, only the *Bacillus* spp., *Serratia marcescens*, and varieties of *S. faecalis* killed a relatively high percentage of the larvae to which they were fed (Table 5). The other pathogenic isolates killed, on the average, less than 20% of the animals in the feeding trials.

If these microorganisms operate at such low levels of pathogenicity in natural populations, particularly sparse populations, it seems likely that individually they contribute little, if at all, to the population dynamics of this insect. On the other hand, the fact that such a wide variety of bacteria exist that are potentially pathogenic for this insect leads us to believe that, under conditions of high host densities, the collective mortality caused by these bacterial species could well contribute significantly to the population dynamics of this insect.

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