U. S. FOREST SERVICE RESEARCH NOTE NE-50

1966

N

FOREST SERVICE, U. S. DEPT. OF AGRICULTURE, 6816 MARKET STREET, UPPER DARBY, PA.

E

BACTERIA OF LIVING AND DEAD LARVAE OF *PORTHETRIA DISPAR* (L.)

A preliminary study of the bacteria associated with living and dead larvae of the gypsy moth *(Porthetria dispar* (L.)) was undertaken to determine what types of micro-organisms may be associated with disease in this insect. Specific objectives of this study were to enumerate the types of aerobic bacteria, and if possible to further elucidate the role of disease-causing micro-organisms in the population dynamics of this insect (Campbell 1963). In addition, information was taken to determine if there was a gross relationship between the habitat of the insect and the microflora encountered.

Although many bacterial genera and species from other insects have been described in qualitative studies, only scant information is available about the resident or transitory population of the gypsy moth. In the first phase of this investigation, Dubois[1] used media containing plant and insect tissues to isolate the maximum number of aerobic bacterial types present. None of the devised media was found to be better than trypticase soy agar, which is employed routinely for the cultivation of bacteria.

The authors, along with other insect pathologists, realize that the framework of any survey is based upon the availability and suitability of selective media. In this sense the experimental design was restrictive. By employing trypticase soy agar aerobically, they eliminated the possibility of isolating anaerobes and other fastidious micro-organisms. Thus this investigation dealt only with bacteria capable of growth under the stated conditions.

[1]Normand R. Dubois. U. S. Forest Serv., NE. Forest Exp. Sta., Forest Insect Res. Div. Quart. Rep. July-Sept. 1961. 14 pp., New Haven, Conn.

Materials and Methods

Cultures. — Collections of gypsy moth larvae were made in Schenectady County, New York, in the summers of 1961, 1962, and 1963. A total of 345 isolates were obtained from 196 dead instar IV-VI larvae, 58 living instar V larvae, and 17 dead larvae that had been parasitized (containing the parasite maggot).

Isolation procedures. — Of the 345 bacterial isolates investigated, 251 were obtained from the dead larvae, 69 from the living larvae, and 25 from the parasitized larvae.

Each dead larva was surface-treated for 5 minutes in a 0.5-percent Clorox solution that contained 0.01 percent Tween 80.[2] The larva was rinsed five times with sterile water, and 1 ml. of the final rinse was plated on trypticase soy agar (TSA) to assure sterility of the larval surface. Each treated larva was macerated with a sterile glass rod in 1 ml. of sterile water. A loopful of the crushed material was streaked on TSA and incubated aerobically at 28°C. for 24 hours.

After visible growth had occurred on the plates, all colonies that appeared different were transferred to TSA slants. Stock cultures were maintained on TSA at 4°C.

Site descriptions. — In an effort to determine if bacterial isolations were related to a particular ecological area, 55 dead and 54 living larvae of those mentioned above were collected in three forest sites for which pertinent descriptive data were taken (Campbell 1963).

Site E was primarily a dry site with well-drained soil. White oak (*Quercus alba* L.) was the predominant tree species, with red oak (*Quercus rubra* L.) and pignut hickory (*Carya glabra* (Mill.) Sweet) next in abundance. The undergrowth was sparse.

Site F was along a well-drained ridge, neither wet nor dry. Red and white oak predominated, with an understory of witch hazel (*Hamamelis virginiana* L.) and hophornbeam (*Ostrya virginiana* (Mill.) K. Koch).

Site H, classified as a wet site, had 8 inches of organic muck lying directly over an impermeable layer. Swamp white oak (*Quercus bicolor* Willd.) and red maple (*Acer rubrum* L.) were the predominant tree species, with little shrub cover.

Identification of isolates. — Colonial, morphological, and physiological characteristics of the microbial isolates were determined by standard

[2]Mention of a particular commercial product should not be construed as an endorsement by the U. S. Department of Agriculture or the Forest Service.

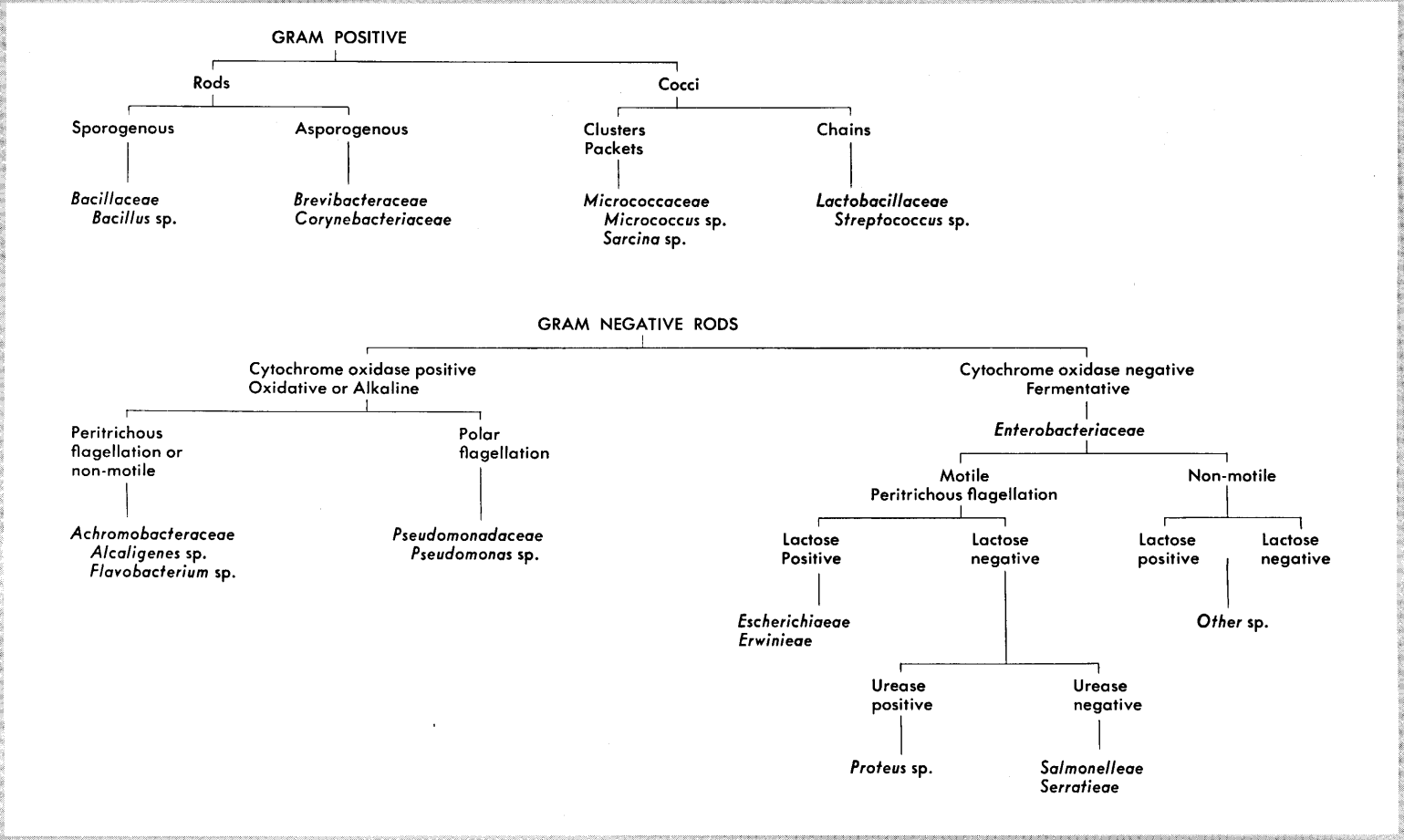

Figure 1. — General scheme for identifying bacterial isolates.

bacteriological techniques as described in the *Manual* of the American Society of Microbiologists (1957), unless otherwise indicated.

Colonial characteristics were determined from 48-hour cultures on TSA plates or slants.

Morphological observations were made by using bright-field and phase-contrast illumination. Motility and cell arrangement were determined from hanging-drop preparations of 12-hour trypticase soy broth cultures. Flagellar arrangement was detected according to the method of Leifson (1951). Gram stains were made according to the method of Kopeloff and Beerman. Endospore stains were made by steaming cells with 5 percent aqueous malachite green and counterstaining with 1-percent aqueous safranin.

The anaerobic fermentation of glucose was detected by using a method of Hugh and Leifson (1953). Lactose fermentation was detected in Phenol red lactose broth (BBL). Cytochrome oxidase was determined using Kovacs' method (1956). Urease activity at 37°C. was detected in urea broth (BBL).

Bacterial types were identified according to a stepwise scheme outlined in figure 1. The investigators are well aware of the shortcomings of such a procedure, but for the purpose of this survey it was deemed adequate. No attempt was made to identify the isolates according to species, except where noted.

Results and Discussion

Bacterial types isolated from dead, living, and parasitized gypsy moth larvae are shown in table 1. No striking difference appeared in frequency of isolation of a particular bacterial group between living and dead larvae except in the case of the family *Enterobacteriaceae.* Of the isolates from dead larvae, 26.7 percent were members of this family; whereas only 1.4 percent of the isolates from living larvae were members of this group. The significance of this information is not clear, but there is a possibility that members of this family may be responsible for some disease in the larvae. *Proteus myxofaciens* n. sp. is a member of this group and might be associated with disease in the insect, although this microbe in itself is not pathogenic for gypsy moth larvae.[3]

Members of the genus *Bacillus* accounted for 44.9 and 43.4 percent of the isolations from living and dead larvae, respectively. Four cultures of

[3] Podgwaite, John D. PROTEUS MYXOFACIENS N. SP. ISOLATED FROM GYPSY MOTH LARVAE. M.S. Thesis, University of Connecticut, 58 pp. 1965.

Table 1. — Bacterial isolations from gypsy moth larvae

Organism	Number of isolates from larvae				Percent of total isolations			
	Dead	Living	Para-sitized	Total	Dead	Living	Para-sitized	Total
Pseudomonadaceae	5	1	0	6	2.0	1.4	0	1.7
Pseudomonas sp.	5	1	0	6	—	—	—	—
Achromobacteraceae	10	1	0	11	4.0	1.4	0	3.2
Alcaligenes sp.	8	1	0	9	—	—	—	—
Flavobacterium sp.	2	0	0	2	—	—	—	—
Enterobacteriaceae	67	1	17	85	26.7	1.4	68.0	24.6
Escherichieae	14	1	8	23	—	—	—	—
Paracolobactrum sp.	7	0	0	7	—	—	—	—
Other sp.	7	1	8	16	—	—	—	—
Proteeae	48	0	8	56	—	—	—	—
P. myxofaciens	16	0	0	16	—	—	—	—
Providence strains	12	0	4	16	—	—	—	—
Other sp.	20	0	4	24	—	—	—	—
Erwinieae, Serratieae, Salmonelleae	5	0	1	6	—	—	—	—
Micrococcaceae	14	5	1	20	5.6	7.2	4.0	5.8
Micrococcus sp.	12	4	1	17	—	—	—	—
Sarcina sp.	2	1	0	3	—	—	—	—
Brevibacteriaceae, Corynebacteriaceae	10	7	0	17	4.0	10.1	0	4.9
Lactobacillaceae	36	23	6	65	14.3	33.3	24.0	18.8
Streptococcus sp.	24	17	6	47	—	—	—	—
S. faecalis	12	6	0	18	—	—	—	—
Bacillaceae	109	31	1	141	43.4	44.9	4.0	40.9
B. thuringiensis types	4	0	0	4	—	—	—	—
Other Bacillus sp.	105	31	1	137	—	—	—	—
Total	251	69	25	345	100.0	100.0	100.0	100.0

a crystalliferous *Bacillus*, pathogenic for gypsy moth larvae, were isolated and will be reported elsewhere.

The other major group of bacterial isolates was of the genus *Streptococcus*. Certain motile pigmented streptococci from this survey have already been reported as potential pathogens by Cosenza and Lewis (1965). These micro-organisms may be responsible for natural mortality in gypsy moth populations.

Sixty-eight percent of the isolates from parasitized larvae were members of the family *Enterobacteriaceae,* and 24 percent were streptococci. Any attempt to associate these groups with parasitization, on the basis of the data available, would be premature.

Table 2 lists some of the same bacterial isolates shown in table 1 that were isolated from larvae for which we had specific site information. A

Table 2. — Bacteria isolated from larvae associated with site information

Organism	Living larvae from site —				Dead larvae from site —				Total bacterial types per site from site —		
	E	F	H	Total	E	F	H	Total	E	F	H
Pseudomonadaceae	0	0	1	1	0	1	2	3	0	1	3
Pseudomonas sp.	0	0	1	1	0	1	2	3	—	—	—
Achromobacteraceae	0	0	0	1	1	1	1	3	2	1	1
Alcaligenes sp.	1	0	0	1	1	0	1	2	—	—	—
Flavobacterium sp.	0	0	0	0	0	1	0	1	—	—	—
Enterobacteriaceae	0	1	0	1	7	7	8	22	7	8	8
Escherichieae	0	1	0	1	4	2	1	7	—	—	—
Paracolobactrum sp.	0	0	0	0	0	1	1	2	—	—	—
Other sp.	0	1	0	1	4	1	0	5	—	—	—
Proteeae	0	0	0	0	3	5	7	15	—	—	—
P. myxofaciens	0	0	0	0	1	2	3	6	—	—	—
Other sp.	0	0	0	0	1	3	2	6	—	—	—
Erwinieae											
Serratieae	0	0	0	0	0	0	0	0	—	—	—
Salmonelleae											
Micrococcaceae	2	1	2	5	2	0	1	3	4	1	3
Micrococcus sp.	2	1	1	4	2	0	1	3	—	—	—
Sarcina sp.	0	0	1	1	0	0	0	0	—	—	—
Brevibacteriaceae											
Corynebacteriaceae	4	2	0	6	2	1	0	3	6	3	0
Lactobacillaceae	12	2	3	17	7	2	2	11	19	4	5
Streptococcus sp.	12	2	3	17	6	1	1	8	—	—	—
S. faecalis	0	0	0	0	1	1	1	3	—	—	—
Bacillaceae	6	10	15	31	11	3	9	23	17	13	24
Bacillus sp.	6	10	15	31	11	3	9	23	—	—	—

sufficient number of isolates were not available to ascertain if there were any significant differences in the types of bacteria isolated from larvae collected in the different sites. It appeared (table 2) that only one group, the streptococci, differed in frequency of isolation from different sites, most of the streptococcal isolates being from larvae collected in site E.

Literature Cited

American Society of Microbiologists.
1957. MANUAL OF MICROBIOLOGICAL METHODS. 305 pp. McGraw-Hill Book Co., Inc., New York.

Campbell, Robert W.
1963. THE ROLE OF DISEASE AND DESICCATION IN THE POPULATION DYNAMICS OF THE GYPSY MOTH *Porthetria dispar* (L.) (Lepidoptera:Lymantriidae.) Can. Entomol. 95: 426-432.

Cosenza, Benjamin J., and Franklin B. Lewis
1965. OCCURRENCE OF MOTILE, PIGMENTED STREPTOCOCCI IN LEPIDOPTEROUS AND HYMENOPTEROUS LARVAE. J. Invert. Pathol. 7: 86-91.

Hugh, R., and E. Leifson.
1953. THE TAXONOMIC SIGNIFICANCE OF FERMENTATIVE VERSUS OXIDATIVE METABOLISM OF CARBOHYDRATES BY VARIOUS GRAM NEGATIVE BACTERIA. J. Bacteriol. 66: 24-26.

Kovacs, N.
1956. IDENTIFICATION OF *Pseudomonas pyocyanea* BY OXIDASE REACTION. Nature [London] 178: 703.

Leifson, Einar.
1951. STAINING, SHAPE, AND ARRANGEMENT OF BACTERIAL FLAGELLA. J. Bacteriol. 62: 377-389.

— JOHN D. PODGWAITE and BENJAMIN J. COSENZA[4]

■

[4]The authors are, respectively, microbiologist, Northeastern Forest Experiment Station, West Haven, Conn.; and assistant professor, Department of Bacteriology, University of Connecticut, Storrs, Conn.