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Russell J. Beers
Iowa State University

William R. Lockhart
Iowa State University

Earle S. Raun
Iowa State University

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Some Characteristics of Bacteria Isolated From Diseased Larvae of the European Corn Borer

By RUSSELL J. BEERS, WILLIAM R. LOCKHART and EARLE S. RAUN

Abstract. A large proportion of the bacteria isolated from diseased corn borer larvae are pleomorphic, with rods, diplococci, filaments and various transition forms occurring in many cultures. Gram reaction is negative, although the coccoid forms show a tendency to retain gram positivity. In morphology and biochemical characteristics, these isolates resemble certain entomogenous bacteria described by earlier workers, but are similar also to a group of bacteria from human sources which have been designated as members of the tribe Mimeae DeBord. Further studies have been initiated to determine the pathogenicity and the proper taxonomic position of these cultures.

A variety of microorganisms, including protozoa, fungi, viruses and many types of bacteria, have been isolated in the past from diseased insects, both adults and larvae (Steinhaus, 1949). Steinhaus (1952) reported the isolation of 91 strains of bacteria from a group of 274 diseased larvae of the European corn borer, *Pyrausta nubilalis* Hübner, of which "the great majority" were small gram negative rods. Of 48 of these cultures selected for detailed study, 22 proved to be enterobacteria, and 15 of the latter showed the general characteristics of *Aerobacter cloacae* Jordan. The remainder were scattered among three other genera: *Pseudomonas*, *Alcaligenes* and *Achromobacter*.

A large proportion of the bacteria isolated in our laboratories from diseased corn borer larvae appear to be aberrant enterobacteria (Raun, Lockhart and Beers, 1959). The present report will describe preliminary observations on this group of bacteria.

PROCEDURES

Cultures were maintained on slants of nutrient agar, and optimum growth temperatures were determined in nutrient broth and on nutrient agar slants. Gram stains were made, and determinations of indole formation, reduction of nitrate, acidity to methyl red (M.R.), acetylmethylcarbinol production (Voges-Proskauer test, V.P.), utilization of citrate as sole source of carbon, hydrogen sulfide production (H₂S), and reaction in litmus milk were carried out by conventional methods (Society of American Bacteriologists, 1957).

RESULTS

All the cultures grew well both in nutrient broth and on agar slants at temperatures ranging from 25°C. to 37°C., with only minor

differences of doubtful significance. A few lagged behind the others after 24 hours of incubation; after 48 hours these differences were no longer apparent. The biochemical tests were carried out on cultures incubated at 30° C.

Some of the cultures consisted of small gram negative rods only, others contained coccoidal cells with a tendency to occur in pairs and showing some tendency to retain the gram positive character, and still others seemed to consist of a mixture of rods and cocci with what can only be described as intermediate forms. A few of the latter cultures also contained some long, gram negative filamentous cells. On the basis of these characteristics, the cultures were divided into three groups:

Group I. Cultures consisting of gram negative rods.

Group II. Cultures consisting of coccoidal cells.

Group III. Cultures containing both rod and coccoidal forms.

The results of the biochemical tests are shown in Table 1. Few

Table 1

Biochemical Characteristics of Bacteria Isolated from Corn Borer Larvae

Group	Total Number of Cultures	Number of Cultures Showing Positive Reaction					
		Indole	Nitrate	M.R.	V.P.	Citrate	H ₂ S
I	6	1	6	1	4	4	0
II	6	1	3	2	1	2	0
III	8	1	8	1	8	8	0

of the cultures showed any pronounced tendency to reduce litmus; Group III was slightly the weakest in this respect. Nearly all fermented the lactose in milk (with Group I slightly slower than the others), though about half were slow fermentors. One Group I culture showed strong ammonification. Curd formation was weak except in Group III; the same was true of peptonization.

DISCUSSION

Extensive experimental clarification will be required to determine the relationships among these cultures of various morphological types. Many pathogenic bacteria are notoriously pleomorphic, developing upon laboratory cultivation a morphology which is quite different from that displayed at the time of initial isolation from the diseased host. Bacteria which, like our isolates, are coccoid upon initial isolation but revert to gram negative rods after cultivation, were isolated from insects by various workers in the early 1900's. The generic name *Coccobacillus* was used at one time to designate bacteria of this type. Although many of the species so described were suspected of being pathogenic for corn borers or other Lepidop-

tera, attempts to use them for purposes of biological control were largely abortive since the pathogenicity did not seem to be retained when cultures were maintained on laboratory media (Steinhaus, 1949). Most workers today tend to regard these organisms as aberrant enterobacteria; many of the cultures originally identified as *Coccobacillus* are considered identical with *Aerobacter cloacae* (Steinhaus, 1949; Lysenko, 1958).

Bacteria with morphological characteristics strikingly similar to the corn borer strains described here are frequently isolated from human sources, where they may be responsible for certain infections. These human strains could be considered a group of morphologically similar but otherwise unrelated bacteria, some of which may be enterobacteria (Henriksen, 1952). However, it has been suggested that this entire group of organisms be recognized as a separate entity, designated as the tribe Mimeae (DeBord, 1942).

Strains described in the recent literature as "Mimeae-like" are notable for the rarity of indole positive cultures, and of strains which are either M.R. positive or V.P. positive. Ability to reduce nitrate is variable within the group. Lysenko (1958) diagnosed a group of 8 insect strains as: *Aerobacter cloacae* (5 strains), *Serratia marcescens* (2 strains), and *Proteus morganii* (1 strain). All these except the *Proteus* are indole negative and V.P. positive, and all except the *Proteus* and one of the *Aerobacter* strains are M.R. negative. Among the 20 such cultures studied in our laboratory, indole production is also quite rare (3 strains), as is failure to reduce nitrate (2 strains). Only 4 strains are methyl red positive, while acetyl-methylcarbinol production and citrate utilization are variable (13 and 14 strains, respectively). All our cultures are H₂S negative, as were all Lysenko's strains except the *Proteus*.

Further study will be required to determine the pathogenicity of these organisms, and to learn whether isolates from humans and from insects are closely related (possibly meriting their inclusion in the single tribe Mimeae), or whether they are a heterogeneous group with morphological similarities which are quite coincidental. Pathogenicity is ordinarily rather specific, and insect pathogens are not known to infect other animals, but it is not unusual to find that bacteria of quite different pathogenicity have close taxonomic relationships in other respects. Due to the confusion regarding the proper classification of these bacteria, neither the germs *Coccobacillus* nor the tribe Mimeae is recognized in the current (7th) edition of *Bergey's Manual of Determinative Bacteriology*, although both have been included provisionally in earlier editions.

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DEPARTMENTS OF BACTERIOLOGY AND
ZOOLOGY AND ENTOMOLOGY
IOWA STATE UNIVERSITY
AMES, IOWA