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A Strain of *Serratia marcescens* Pathogenic for Larvae of *Lymantria dispar*: Characterization

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A gram-negative bacillus, pathogenic for gypsy moth larvae, was characterized culturally, morphologically, and physiologically as a member of the *Serratia* group of the family Enterobacteriaceae. The microorganism lacked the pigmentation characteristic of the group but was generally distinguished from closely related members of the family by its inability to produce gas from glucose, inositol, glycerol, and cellobiose; its rapid liquefaction of gelatin; and its failure to ferment raffinose or arabinose. The microorganism displayed lecithinase, deoxyribonuclease, and chitinase activity. The percentage of G + C in DNA from this bacterium was within the range reported for known strains of *Serratia marcescens*.

INTRODUCTION

The gypsy moth, *Lymantria dispar,* is a significant defoliator in the northeastern United States and presents a definite threat to hardwood forest stands and forested recreational areas (Corliss, 1955; Perry, 1955; Turner, 1969). Attempts to control this pest with biological agents have met with limited success mainly because of application problems (Lewis and Connola, 1966) and a lack of knowledge concerning fundamental relationships between the insect and its pathogens.

Studies initiated to assess the relative importance of various disease agents in natural gypsy moth populations (Podgwaite and Campbell, 1970; Campbell and Podgwaite, 1971) revealed the presence of many pathogenic microorganisms (Podgwaite and Campbell, 1972). One of these, a gram-negative bacillus, was found to be highly pathogenic for early-instar larvae. Studies of this microorganism were initiated with the following objectives: (1) to characterize it, (2) to assess its pathogenicity, (3) to investigate its mechanism (s) of pathogenicity, and (4) to assess its potential as a biological control agent. The present article is a report on its characterization.

MATERIALS AND METHODS

The microorganism described here, hereafter referred to as 922A, was isolated from dead fourth-instar larvae of *Lymantria dispar* collected in a dense population.

The colonial, morphological, and physiological characteristics of the microorganism were determined by standard bacteriological methods according to the "Manual of Microbiological Methods" (Society of American Bacteriologists, 1957) unless otherwise indicated. When it became apparent that the pathogenic isolate was a member of the Enterobacteriaceae, the keys and procedures described by Edwards and Ewing (1972) and Carpenter et al. (1966) were used in its identification. Incubation of all media, unless otherwise indicated, was at 37°C.

Three cultures of *Serratia marcescens* were used for comparative purposes. One was a typical red strain from the culture collection of the Microbiology Department, the University of Connecticut; and the other two, ATCC 13880 (*Serratia marcescens*) and ATCC 8101 (*Serratia marcescens* subsp. *kiliensis*), were from the American Type Culture Collection, Rockville, Maryland. All cultures were transferred monthly on Trypticase soy agar (TSA) (BBL) slants and kept at 4° C.

Cultural characteristics. Colonial characteristics were determined from 24- or 48-hr cultures on TSA plates or slants and from Trypticase soy broth (TSB) (BBL) cultures.

Morphology. Morphological observations were made by using bright field, phase contrast, and electron microscopy. Motility and cellular arrangement were determined from wet-mount preparations of 12-hr TSB cultures. Flagellar arrangement was determined from platinum-shadowed cell preparations examined with the aid of an RCA EMU 4A electron microscope at 50 kv.

Physiological characteristics. All physiological tests were repeated at least three times to detect variability of cultures. All subcultures used for physiological tests were examined at 1, 2, 3, 5, 7, 14, and 30 days.

The oxidase test was performed according to Kovacs (1956) and Gaby and Hadley (1957). The medium of Hugh and Leifson (1953) was used to test the ability of 922A to ferment glucose anaerobically. DNase activity was detected by flooding 24-hr DNase agar (BBL) plate cultures with 1 N HCl and then examining them for zones of clearing adjacent to colonies.

Lecithinase activity was determined at 24°, 30°, and 37° C on egg yolk agar plates (Difco) after a 48-hr incubation period. Positive cultures exhibited a turbid zone surrounding the colonies.

The ability of 922A to degrade chitin was tested in a medium described by Monreal and Reese (1969). The medium was (in grams/liter): chitin, 15; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3; KH_2PO_4, 1.36; *p*H 7.5. Inoculated media were incubated aerobically with shaking at 30° C for 7 days. After the incubation period, the remaining insoluble chitin was removed, washed, dried, and weighed; and the amount degraded was determined as a percentage of the starting material.

DNA studies. For DNA extraction, cells of 922A were grown in 5-liter batches of TSB at 37° C and harvested in the logarithmic growth phase (14 hr). DNA was extracted from 3 g of wet-packed cells, according to the method of Marmur (1961).

The base composition of extracted DNA was estimated from its thermal denaturation temperature (T_M) (Marmur and Doty, 1962) by using the relationship percentage of G + C = 2.44 (T_M − 69.4) (Deley, 1970) where T_M is in ° C and G + C is the mole percentage of guanine plus cytosine. The base composition of the DNA was also estimated from its uv spectrum by the method of Fredericq et al. (1961).

RESULTS

Forty-eight-hour colonies of isolate 922A on TSA plates were circular (2–3.5 mm), with an undulate edge, umbonate, smooth, opaque, glistening, and butyrous. Growth on 48-hr TSA slants was filiform and on TSB was characterized by the formation of a surface ring and a granular sediment. The University of Connecticut strain and ATCC 13880 displayed the red pigmentation characteristic of the species, whereas ATCC 8101 and 922A did not.

Isolate 922A was gram-negative. Wet mounts of young TSB cultures revealed motile cells with rounded ends arranged predominantly in singles and in pairs but also occasionally in short chains of three or four cells. Flagellar arrangement was peritrichous (Fig. 1) with from three to thirteen flagella per cell. The predominant arrangement was four or five flagella per cell. The average cell size, determined from electron micrographs of older cells, was 1.6 × 1.2 μm.

Isolate 922A was mesophilic, displaying

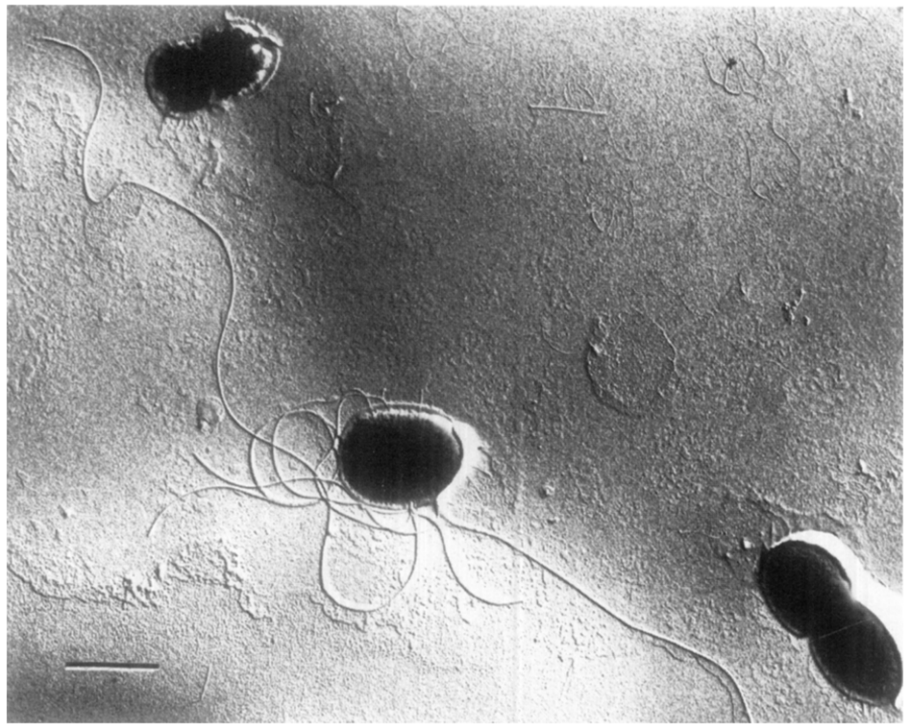

FIG. 1. Electron micrograph of 922A showing peritrichous flagellar arrangement. Shadowed with Pt. Bar = 1 μm.

TABLE 1
Biochemical Characteristics of Isolate 922A Compared with Known *Serratia* spp

Test	Reaction[a] 922A	UC	13880	8101
Motility	+	+	+	+
Cytochrome oxidase	−	−	−	−
Reaction in Hugh's medium	F	F	F	F
Nitrate reduction	+	+	+	+
Indole	−	−	−	−
Methyl red	−	−	−	$+^{w}$
AMC production	+	$+^{w}$	$+^{w}$	−
Citrate	+	+	+	+
H_2S	−	−	−	−
Growth in KCN	+	+	+	+
Gelatin liquefaction	+	+	+	+
Urease	−	−	−	−
Phenylalanine deaminase	−	−	−	−
Ammonium salts glucose agar	+	+	+	+
Malonate	−	−	−	−
Mucate	−	−	−	$+^{w}$
Lysine decarboxylase	+	+	+	+
Arginine dihydrolase	−	−	−	−
Ornithine decarboxylase	+	+	+	+
Pigment	−	+	+	−

[a] +, Positive reaction or characteristic; −, negative reaction or characteristic; $+^{w}$, weakly positive reaction; F, fermentative.

TABLE 2
Carbohydrate Utilization by 922A and Known *Serratia* spp

Substrate	Reaction[a] 922A	UC	13880	8101
Glucose	+	+	+	+
Lactose	$+^{lw}$	$+^{lw}$	$+^{lw}$	$+^{lw}$
Sucrose	+	+	+	+
Maltose	+	+	+	+
Mannitol	+	+	+	+
Dulcitol	−	−	−	−
Rhamnose	−	−	−	−
Arabinose	−	−	−	−
Inositol	$+^{w}$	$+^{w}$	$+^{w}$	$+^{w}$
Xylose	−	−	−	−
Raffinose	−	−	−	−
Sorbitol	+	+	+	+
Salicin	+	+	+	+
Adonitol	$+^{w}$	$+^{w}$	$+^{w}$	−
Trehalose	+	+	+	+
Cellobiose	−	$+^{lw}$	$+^{lw}$	−
Glycerol	+	+	+	$+^{w}$
Gas from glucose	−	−	−	−
Gas from inositol	−	−	−	−
Gas from glycerol	−	−	−	−
Gas from cellobiose	−	−	−	−

[a] +, Acid reaction in 1 or 2 days; −, negative reaction; $+^{w}$, weak acid reaction; $+^{lw}$, late and weak acid reaction.

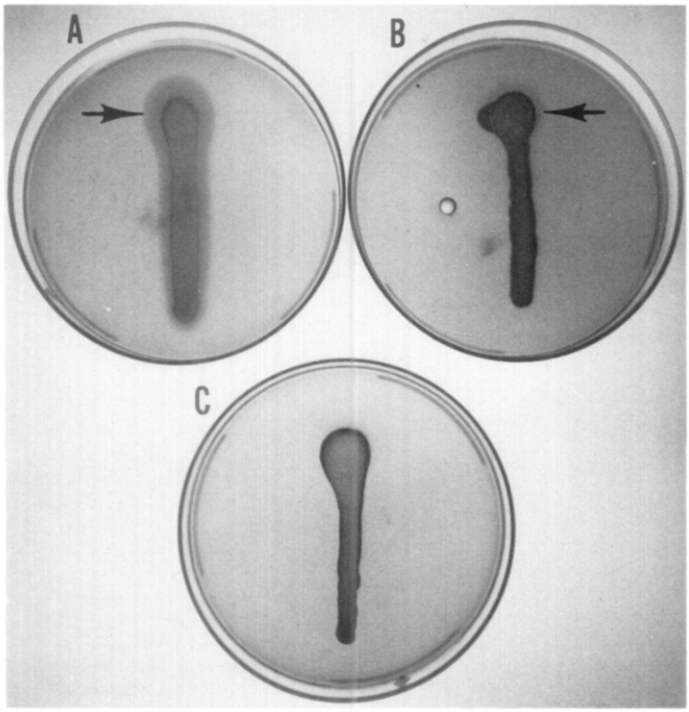

FIG. 2. Zones of lecithinase activity (arrows) of 922A incubated at different temperatures on EY agar: (A) 37°C (B) 30°C; (C) 24°C.

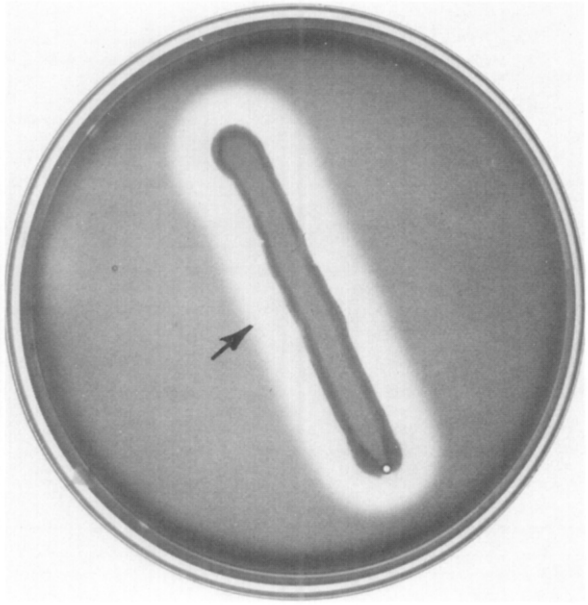

FIG. 3. Culture of 922A displaying deoxyribonuclease activity (arrow) on DNase agar.

growth within the range 10–37°C and growing optimally between 24° and 37°C. It did not grow at 5° or 45°C. The oxygen relation of the microorganism was determined to be facultative; it had optimal growth when incubated in air, limited growth under reduced O_2 tension, and no growth under conditions of strict anaerobiosis. The microorganism grew within the *p*H range 5.5–10 with optimum growth between 7.5 and 9.0.

The biochemical characteristics of 922A are compared to those of known *Serratia* spp. in Table 1. Carbohydrate utilization by 922A and known *Serratia* spp. is shown in Table 2. Isolate 922A was found to exhibit considerably higher lecithinase activity at 37°C than at 30° or 24°C (Fig. 2). The potent deoxyribonuclease activity of the microorganism is shown in Figure 3.

The ability of the microorganism to degrade chitin was demonstrated by the loss of 40–50% of this material in culture after a 7-day incubation period. No attempts were made to isolate or purify the enzyme(s) responsible, or to determine its activity in vivo.

The thermal denaturation temperature of native DNA samples isolated from 922A was 94.5 ± 0.5°C. A typical melting curve is shown in Figure 4. The corresponding G + C content was determined to be 60.2–62.5%, calculated by the formula of Deley (1970). The ratio E_{260}/E_{280} of 922A DNA at *p*H 3.0 was found to be 1.23. According to Fredericq et al. (1961), this corresponds to a G + C content of 59.0%, which is consistent with that determined by thermal denaturation.

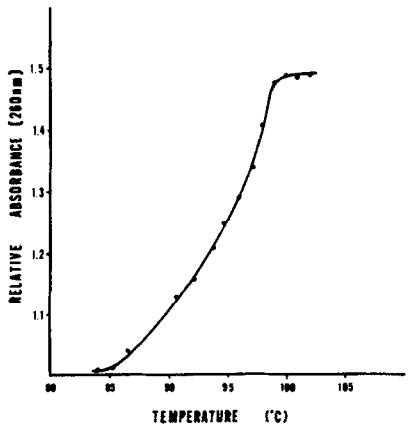

FIG. 4. Thermal denaturation curve of native DNA from 922A. T_M = 94.5°C.

DISCUSSION

The bacterial pathogen described here is culturally, morphologically, and physiologically similar to members of the *Serratia* group of the Enterobacteriaceae. "Bergey's Manual of Determinative Bacteriology" (Buchanan and Gibbons, 1974) categorizes *Serratia* spp. as gram-negative, motile peritrichously flagellated rods that typically produce characteristic red pigments and DNase.

Isolate 922A lacks the pigmentation characteristics of the group but may be generally distinguished from closely related members of the Enterobacteriaceae by its failure to produce gas from glucose, inositol, glycerol, and cellobiose; its rapid liquefaction of gelatin; and its failure to ferment raffinose or arabinose. It is differentiated from *Klebsiella* by its motility and ornithine decarboxylase activity; from *Enterobacter cloacae* by its late and weak acid reaction in lactose, its failure to utilize malonate, and its lysine decarboxylase activity; from *Enterobacter aerogenes* by its failure to produce gas from fermentable carbohydrates as well as its late reaction in lactose; from *Enterobacter hafnia,* again by its lack of gas production and positive reaction in sorbitol and gelatin liquefaction; from *Serratia (Enterobacter) liquefaciens* by its lack of gas production and failure to ferment raffinose and arabinose; and from *Proteus* by its lack of urease and phenylalanine deaminase activity.

Additionally, 922A exhibited lecithinase activity. Fourteen of sixteen *Serratia* spp. tested by Esselman and Liu (1960) displayed this activity, whereas other members of the Enterobacteriaceae tested did not.

Furthermore, 922A displayed potent deoxyribonuclease activity. Martin and Ewing (1967) tested 218 *S. marcescens* strains and found that all exhibited DNase activity. Sixty cultures of *Serratia (Entero-*

bacter) liquefaciens also displayed this activity but to a lesser degree. The only other members of the Enterobacteriaceae showing this activity were cultures of *Proteus vulgaris* and strains of *Pectobacterium.*

The average melting temperature of 94.5° C of DNA from the bacterium is within the range 93.3–95.9°C reported by Colwell and Mandel (1965) for 33 strains of *S. marcescens.* This corresponds to a G + C content of 61.2%, using Deley's correction (1970) of Marmur and Doty's original equation (1962). Thus, the taxonomic evidence gathered indicates that 922A is a nonchromogenic variant of *S. marcescens.*

Although nonchromogenic varieties of *S. marcescens* have been reported to be pathogenic for certain insects (Bucher, 1959; Lawson, 1959; Stevenson, 1959), this is the first report of such a variety infecting gypsy moth larvae.

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