

A new species of *Proteus* isolated from larvae of the gypsy moth *Porthetria dispar* (L.)¹

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Characteristics of a slime-producing bacterium isolated from living and dead gypsy moth larvae were determined. The bacterium was found to be a motile, gram-negative rod, which fermented glucose, but not lactose. It was oxidase-negative, hydrolyzed urea, deaminated phenylalanine and produced H₂S. These characteristics are common to several members of the genus *Proteus*. In addition to the unique feature of slime production, a biochemical comparison with other members of the genus showed that it differed markedly from described species of *Proteus*; thus the new species *Proteus myxofaciens* was proposed.

INTRODUCTION

Since the introduction of the genus *Proteus* by Hauser (1885), observations of its biochemical activities have resulted in the formation of a well-defined group. For example, Smith (1894) noticed that *Proteus* spp. fermented glucose and sucrose with acid and gas production, but failed to ferment lactose. Later, Wenner and Rettger (1919) divided their cultures into two species, *P. vulgaris* and *P. mirabilis*, on the basis of maltose fermentation. In 1927, Moltke demonstrated rapid decomposition of urea by *Proteus* spp. More recently, Bergey's Manual (1957) divided the genus into five species, *P. vulgaris*, *P. mirabilis*, *P. rettgeri*, *P. morganii*, and *P. inconstans*, which are distinguished primarily by the following physiological characteristics: urea hydrolysis, mannitol and maltose fermentation and indol production.

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Several investigators have identified or described *Proteus* spp. isolated from insects. Brown (1927) reported *P. photuris* from luminous organs of the firefly *Photuris pennsylvanicus* (DeG); Steinhaus (1941) described two species, *P. recticolens* and *P. insectocolens* from the milk bug, *Oncepeltus faeciatius* (Dallas) and Wistreich and Chao (1960) identified *P. rettgeri* isolated from the gut of *Culex tarsalis* (Coquillett). With the exception of *P. rettgeri* these *Proteus* spp. isolated from insects do not resemble any of the above recognized species.

In the present study, a slime-producing bacterium is described which possesses morphological, cultural, and physiological activities of the genus *Proteus*. The isolate has been recovered from living and dead gypsy moth larvae collected in Glenville, New York, during a 3 year population dynamics study of the gypsy moth. It also has appeared in an outbreak of gypsy moth larvae in Colchester, Connecticut.

METHODS

The isolation procedures used in this study were those reported previously by Cosenza and Lewis (1965). Morphological, cultural and physiological characteristics described in the Manual of Microbiological Methods (1957) were employed to determine the taxonomic position of the bacterium. Cells from a 18 hr nutrient agar slant were gram-stained and morphological observations were made with bright field and phase contrast microscopy. Flagella stains were prepared according to the method of Leifson (1960). Colonial and cultural characteristics were observed in Trypticase Soy broth (TSB, Baltimore Biological Laboratories) and Trypticase Soy agar (TSA). Phenylalanine deamination and cytochrome oxidase were determined by the methods of Ewing, Davis and Reavis (1957) and Ewing and Johnson (1960). The decomposition of urea was detected in urea broth (Stuart, van Stratum and Rustigian, 1945). Hydrogen sulfide (H_2S) and indol production were tested in SIM medium (Difco). The indol test was performed with Kovacs' reagent. Glucose fermentation was confirmed by the method of Hugh and Leifson (1953). All cultures were incubated at 28 C and examined at 24, 48, 72 and 120 hr with the exception of the sugar broths (nutrient broth + 0.5% carbohydrate) which were held for two weeks.

RESULTS AND DISCUSSION

During a 3 year population dynamics study of the gypsy moth, an unusual bacterium has been recovered from living and dead gypsy moth larvae collected in Glenville, New York. It has also appeared in an outbreak of gypsy moth in Colchester, Connecticut. This slime-producing isolate was a gram-negative

rod, averaging about $0.8\ \mu$ in width by $2.0\ \mu$ in length. During the early phases of the growth cycle the cells appeared to be "coccobacillary", a term frequently used to describe insect isolates. As the culture aged, the cells became more rod-shaped. A typical flagellated cell is presented in Fig. 1. On TSA plates, the

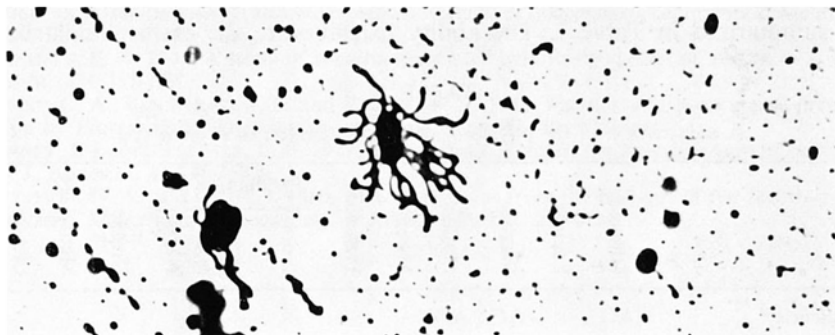


Fig. 1. *Proteus myxofaciens* showing peritrichous flagellation. Leifson stain. 2000 $\times$.

colonies appeared circular, entire, glistening, butyrous and swarmed on a moist agar surface. The unique slime material was formed only in TSB. The viscosity of the slime becomes apparent by viewing the intact slime plug poured from the tube into a petri dish (Fig. 2). The temperature range for growth of the

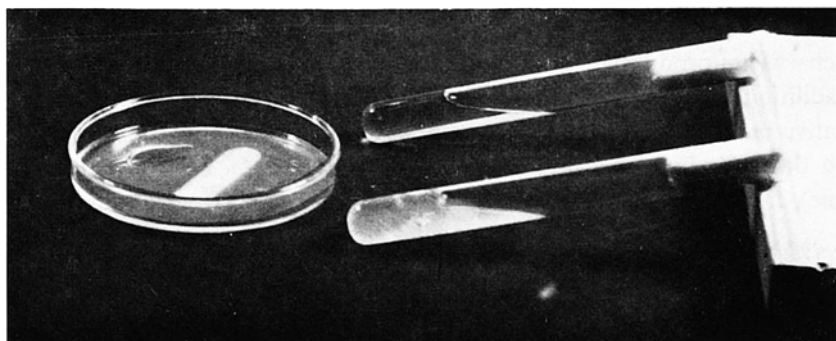


Fig. 2. Uninoculated TSB control (upper), slime production in TSB (lower) and petri dish showing the appearance of an intact slime plug.

slime-producing bacterium was 10 – 45 C with the optimum between 28 C and 37 C. Optimum pH for growth was in the range 7.0 – 8.5 with no growth at pH 4.5 or at 10. *P. myxofaciens* exhibits the following biochemical characteristics of the genus *Proteus*: cytochrome-oxidase-negative, deaminates phenylalanine, ferments glucose with gas, fails to ferment lactose, hydrolyzes urea and produces H_2S .

A comparison of the flagellar arrangements of all known species of the genus and *P. myxofaciens* showed no significant differences. When *Proteus myxofaciens* was grown on a TSA slant, a yellowing of the medium occurred after five days incubation. A similar reaction was observed in cultures of *P. morganii* and *P. mirabilis*. The results of a comparative physiological study of *Proteus* spp. are summarized in Table 1. The ability to elaborate the extracellular slime,

TABLE 1

A comparison of the physiological characteristics of *Proteus* spp.

Species	Glucose	Lactose	Sucrose	Maltose	Mannitol	Trehalose	Production of			Urease	Methyl red	Citrate utilization
							Indol	H ₂ S	AMC			
<i>P. vulgaris</i> ²⁾	AG	-	AG	AG	-	-	+	+	-	+	+	-
<i>P. mirabilis</i> ²⁾	AG	-	-	-	-	-	-	+	-	+	+	-
<i>P. rettgeri</i> ¹⁾	A	-	A ^W	-	A	-	+	-	-	+	+	-
<i>P. inconstans</i> ¹⁾	A	-	A ^W	-	-	AG	+	-	-	-	+	+
<i>P. morganii</i> ²⁾	AG	-	-	-	-	-	+	W	-	+	+	-
<i>P. myxofaciens</i> sp. n.	AG	-	AG	AG	-	AG	-	+	+	+	-	-

¹⁾ Obtained from the Division of Laboratories, State of Connecticut.

²⁾ Stock culture collection Bacteriology Department, University of Connecticut.

AG = Acid and gas, A = Acid, + = Positive, - = Negative, W = Weak.

which was not produced by any other described species, is of particular interest. In addition, failure to produce indol, inability to lower the pH yielding a negative methyl red test and the production of acetyl methyl carbinol (AMC) form the basis for proposing the name *Proteus myxofaciens* sp. n., myxo G. (slime), faciens L. (producing).

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