anomethrin, kadethrin, and *S*-bioallethrin, ten of which are potent insecticides (*1*) or are highly toxic when injected into mammals (*2, 4*).

Specific binding of [^{35}S]TBPS is inhibited by the toxic isomers of each α-cyanophenoxybenzyl pyrethroid and to a greater degree by the *cis* than the *trans* isomers, in agreement with their relative toxicity. Binding is not inhibited by any nontoxic stereoisomer, presumably because the site is stereospecific relative to each chiral center in the pyrethroid. Pyrethroids producing the type 1 syndrome are essentially inactive in this assay with brain synaptic membranes, indicating that their primary action may be elsewhere. The mechanism of toxicity of the cyanophenoxybenzyl pyrethroids therefore appears to involve an interaction with the TBPS-PTX receptor by binding to a closely associated though possibly not identical site in the GABA receptor–ionophore complex.

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A Cellulolytic Nitrogen-Fixing Bacterium Cultured from the Gland of Deshayes in Shipworms (Bivalvia: Teredinidae)

Abstract. *A novel bacterium has been isolated in pure culture from the gland of Deshayes in six species of teredinid bivalves. It is the first bacterium known to both digest cellulose and fix nitrogen, and it is a participant in a unique symbiotic relation with shipworms that may explain how teredinids are able to use wood as their principal food source.*

It has been reported that adult shipworms, wood-boring bivalves of the family Teredinidae, can grow on a diet of wood alone unsupplemented by filter feeding (*1*). However, there is considerable disagreement over the origin of the enzymes necessary for the digestion of cellulose and the means by which these mollusks meet their nitrogen requirements when feeding on wood. We describe here the isolation and purification of a cellulolytic, nitrogen-fixing bacterium from the gland of Deshayes in six species of shipworms: *Lyrodus pedicellatus* (Quatrefages), *Bankia gouldi* (Bartsch), *Teredo navalis* Linnaeus, *Teredo furcifera* Von Martens, *Teredo bartschi* Clapp, and *Psiloteredo healdi* (Bartsch). Its presence in enormous numbers in the gland of Deshayes (Fig. 1a) may account for the ability of shipworms to digest cellulose and to balance their nitrogen budget.

The gland of Deshayes is unique to the Teredinidae. It was discovered more than a century ago by Deshayes (*2*) and subsequently named and described by Sigerfoos (*3*). To date, functions ascribed to this gland have been inferred largely from circumstantial evidence. They include the production of cellulolytic enzymes (*4*) and the synthesis of amino acids (*5*). The gland consists of a brown, irregular mass of tissue lining the

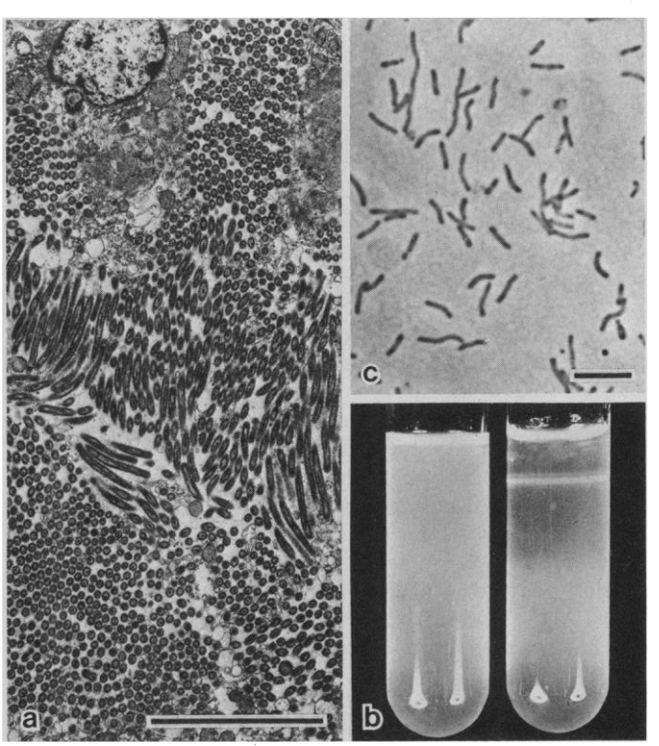

Fig. 1. (a) Transmission electron micrograph of a thin section of the gland of Deshayes from *L. pedicellatus*, showing numerous Gram-negative, rod-shaped bacteria. Scale bar, 5 μm. (b) Bacterial isolate from *L. pedicellatus*, shown growing on colloidal cellulose agar medium (tube on right). Note the distinct band of cells below the agar surface and the disappearance of the colloidal cellulose both above and below this band. The tube at left contains uninoculated medium; colloidal cellulose is uniformly distributed. (c) Phase-contrast photomicrograph of the bacterium isolated from the gland of Deshayes in *L. pedicellatus*. Scale bar, 5 μm.

afferent branchial vein and penetrating the gill lamellae. Two ducts, one for each gill, lie laterally adjacent to the gland in the afferent branchial vein and extend from the posterior to the anterior portion of the gill, where they appear to join the esophagus (*6*). Popham and Dickson (*7*), using transmission electron microscopy, showed in several species of shipworms that the gland contains numerous rod-shaped, Gram-negative bacteria.

To isolate the bacterium from the gland of Deshayes, we first dissected the gland from individual shipworms that had been removed from wooden panels and washed in sterile seawater. The gland and associated gill tissue were then washed several times in sterile seawater and homogenized. Cultures were established by serial dilution in a mineral medium (*8*) supplemented with 0.2 percent agar, with powdered cellulose (Sigmacell type 100; Sigma) as the source of carbon and energy and no source of combined nitrogen. Growth appeared as a sharply defined lens of cells about 1 cm below the agar surface (Fig. 1b). Cells for isolation were removed from the tubes of greatest dilution (typically between 10^7 and 10^8 dilution) and streaked onto a solid medium (0.9 percent agar) made of the mineral base (*8*) with powdered cellulose and ammonium chloride (5 m*M*). Axenic stock cultures were maintained in tubes of basal medium (*8*) containing 0.2 percent agar, with cellulose as the carbon and energy source and no source of combined nitrogen.

Partial characterization indicates that the bacterial isolates from the six species of shipworm are similar and probably represent a single species of bacterium. It is a Gram-negative rigid rod, 0.4 to 0.6 μm wide and 3 to 6 μm long (Fig. 1c), that moves by means of a single polar flagellum (*9*). In stationary phase cultures the cells often become pleomorphic, appearing spiraled or as very long rods. The guanine plus cytosine content of the DNA ranges from 49 to 51 mole percent G+C (*10*).

This bacterium is an aerobic chemoheterotroph that will grow in a simple mineral medium containing seawater and a suitable source of organic carbon. It requires a source of combined nitrogen if vigorously aerated but will fix molecular nitrogen, as documented by the acetylene reduction method (*11*), when grown under microaerophilic conditions. It is obligately marine, requiring elevated concentrations of Na^+, Cl^-, Mg^{2+}, and Ca^{2+} for growth (*12*). To date, growth has been demonstrated by using the following compounds as sole carbon and energy sources: cellulose (Whatman No. 1 filter paper or Sigmacell 100), carboxymethyl cellulose (0.5 percent, weight to volume), cellobiose (0.5 percent), glucose (0.5 percent), fructose (0.5 percent), sucrose (0.5 percent), acetate (0.1 percent), succinate (0.1 percent), and glutamate (0.1 percent). The optimum temperature for growth is 35°C; growth is absent at 39°C. Specific growth rates (*13*), measured at 35°C by using powdered cellulose in the presence or absence of combined nitrogen, are 0.12 and 0.05 per hour, respectively (Fig. 2).

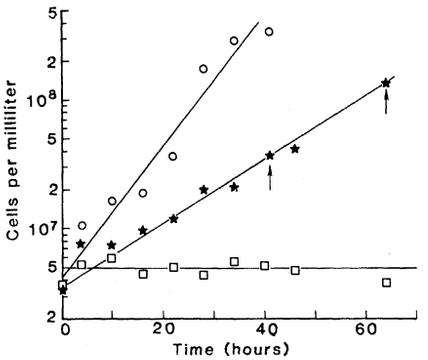

Fig. 2. Growth of bacterial isolate from *L. pedicellatus* at 35°C in an atmosphere of nitrogen plus 1.0 percent O_2 and 500 ppm CO_2 in liquid basal medium (*8*). (□) Control culture containing 5 m*M* ammonium chloride but no carbon source. (○) Culture containing powdered cellulose and 5 m*M* ammonium chloride. (★) Culture containing powdered cellulose but no combined nitrogen. Arrows show when samples were withdrawn for measurement of acetylene reduction (*11*). Ethylene was produced at 23 and 24 nanomoles per milligram of protein per minute at hours 41 and 64, respectively.

These bacterial isolates appear to be unique, fitting into none of the known genera of bacteria. However, before they are formally named it will be necessary to complete their characterization and establish their taxonomic affinities.

Three lines of evidence indicate that the bacterium we isolated is the bacterium present in the gland of Deshayes. First, precautions were taken to minimize contamination during removal of the gland, and subsequent isolations were made from serial dilutions after a 10^7 or 10^8 dilution of gland homogenate. Using these procedures, we were able to repeatedly isolate the same bacterium from a number of different animals of a single species as well as from six different species collected and maintained at different locations. Conversely, we have been unable to isolate the bacterium from either seawater or the normal wood flora. Second, the very uniqueness of the physiology of all the bacterial isolates, that is, the ability to degrade cellulose and fix nitrogen, argues against their being chance contaminants. Finally, the bacterium in the gland of Deshayes is morphologically distinct. The isolates are morphologically similar both to the bacterium in the gland and to each other.

We anticipate that the bacterium will be shown to be the primary source of cellulolytic enzymes in shipworms and that nitrogen fixation by the bacterium will enable these mollusks to meet their nitrogen requirements when growing exclusively on wood. The fact that a single species of bacterium has been isolated from six species of shipworms collected from a number of geographic locations indicates a specific association between this bacterium and members of the Teredinidae and suggests that the same bacterium is present in the gland of Deshayes in other teredinids.

The ability of shipworms to use cellulose is not unique. Cellulolytic activity is widespread in invertebrates (*14*), where it is associated with gut flora or perhaps produced by the animals themselves. However, the gland of Deshayes is the only example of an association between a cellulolytic bacterium and an organ that is not part of the digestive tract. Hence invertebrates that are thought to produce cellulases endogenously should be reexamined.

Nitrogen-fixing bacteria have been shown to occur as part of the gut flora in animals whose diet is rich in carbon but deficient in nitrogen, most notably in termites (*15*) but also in shipworms (*16*). Carpenter and Culliney (*16*) measured acetylene reduction associated with shipworms and isolated a Gram-negative bacterium from the cecum of *L. pedicellatus* that was capable of fixing nitrogen under anaerobic conditions. These studies were not pursued and the bacterium was lost. We have subsequently isolated marine spirilla from the cecal contents of shipworms that are capable of fixing nitrogen under microaerophilic conditions. However, the cecal contents contain very few spirilla, and nitrogen-fixing spirilla can be readily isolated from wood incubated in seawater. This suggests that their presence in the cecum is a consequence of teredinid feeding.

The symbiosis between the bacterium of the gland of Deshayes and shipworms is similar to the association between luminous bacteria and the light organs in fish and some squid (*17*). As in the case of light organs, the gland of Deshayes appears to harbor a bacterium in a pure state. This symbiotic relationship also bears a functional similarity to the nodule-*Rhizobium* symbioses in leguminous plants. We know of no other animal that

harbors a nitrogen fixer as a pure culture in a specialized organ.

The bacterium from the gland of Deshayes possesses two properties, cellulose digestion and nitrogen fixation, that genetic engineers have been trying (unsuccessfully) to combine in a single bacterium. The combination of these properties in this bacterium makes it a candidate for producing single-cell protein from cellulose without the necessity of adding combined nitrogen.

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Cat Scratch Disease: A Bacterial Infection

Abstract. *Histopathologic examination of lymph nodes from 39 patients with clinical and pathological criteria for cat scratch disease revealed delicate pleomorphic Gram-negative bacilli in 34 of the 39 nodes. They were within the walls of capillaries in or near areas of follicular hyperplasia and within microabscesses. They were best seen with the Warthin-Starry silver impregnation stain. Organisms in lymph node sections exposed to convalescent serum from three patients and to immunoperoxidase stained equally well with all three samples. The organisms did not react with hyperimmune sera to* Legionella pneumophila *nor to several species of Rickettsia. These bacilli appear to be the causative agents of cat scratch disease.*

Despite at least 750 reports on cat scratch disease (CSD) over the past 37 years, the etiologic agent of the disease has eluded detection. Clinical, epidemiological, and pathological studies have implicated an infectious agent (*1–5*).

A clinical diagnosis of CSD requires the fulfillment of three of four criteria (*2*): (i) a history of animal (usually a cat or dog) contact with the presence of a scratch or primary dermal or eye lesion (*6*), (ii) a positive CSD skin test, (iii) negative laboratory studies for other causes of lymphadenopathy, and (iv) characteristic histopathology of a biopsied lymph node. A definitive diagnosis will be possible only when the etiologic agent is isolated. Many patients go undiagnosed because the symptoms are atypical. For instance, 10 percent of the patients with otherwise characteristic symptoms report no contact with cats and 35 percent of the patients give no history of a cat scratch.

The Armed Forces Institute of Pathology (AFIP) provides consultation on approximately 50,000 specimens a year, usually to assist when a diagnosis is controversial or unclear. Some of these specimens are from patients suspected of having CSD. In early 1981, we received a lymph node taken from an 11-year-old girl. Although the girl's family had cats, there was no history of a cat scratch. Laboratory findings supported the diagnosis of CSD because organisms failed to grow from this lymph node when cultured on blood agar, chocolate agar, prereduced brucella agar, laked blood agar, phenylethyl alcohol agar, or thioglycollate medium. We stained sections of the node for bacteria, mycobacteria, fungi, and spirochetes. Using a silver impregnation stain we observed many small bacteria that proved to be Gram-negative with a Gram stain modified for tissue. These observations prompted us to search for similar bacteria in lymph nodes from additional patients with CSD.

Between 1 October 1982 and 31 March 1983, 39 lymph nodes with histologic changes of CSD as described by Campbell (*7*) were submitted to the AFIP. These came from 17 states and Europe. In 28 of 39 specimens the pathologist included CSD in the provisional diagnosis. Twenty-seven of 39 patients were children or young adults. Exposure to cats was not mentioned for 28 patients but six patients had been scratched by cats and five others exposed to cats. In 37 of 39 patients, a single cluster of nodes was enlarged. The location of these clusters was as follows: epitrochlear (four patients), axillary (eight), supraclavicular (one), cervical (six), parotid (two), anterior chest wall (three), inguinal (ten), femoral (one), and thigh (two). After the patients' physicians were contacted, skin tests were done on eight patients and all were positive for CSD. The lymph nodes of seven of these patients contained detectable bacteria.

Bacilli were clearly seen with the Warthin-Starry (WS) silver impregnation stain (*8*) in lymph node sections from 29 of 34 patients and with the modified Brown-Hopp's tissue Gram stain (*9*) in 28 of 34 patients. The bacilli were pleomorphic, ranged from 0.3 to 1.0 μm by 0.6 to 3.0 μm, were Gram-negative, and were not acid-fast. They were in the walls of capillaries (Fig. 1, A and B) and in macrophages lining the sinuses in or near germinal centers. Here the bacilli appeared as single organisms or in chains or clumps. In some cross sections of vessel walls the bacilli encircled the lumen. In longitudinal sections, large numbers of bacilli almost obliterated a short segment of vessel wall. Bacilli were also in thrombosed vessels, and in necrotic foci where they were clustered in histiocytes, giving the appearance of intracellular multiplication (Fig. 1C). In some areas of more extensive necrosis the bacilli appeared as single organisms in vacuoles of activated histiocytes or free in the necrotic debris. When the neutrophils were centered within stellate granulomas, bacilli were only rarely observed within neutrophils or free in the necrotic exudate. The bacteria we observed fulfilled the criteria for a pathogenic organism in these lymph nodes: they were in the tissue, they were limited to the areas of reaction, they were intracellular, and