

Coriobacterium glomerans gen. nov., sp. nov. from the Intestinal Tract of the Red Soldier Bug

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A new gram-positive anaerobic bacterium is described on the basis of 89 strains isolated from the intestinal tracts of red soldier bugs (*Pyrrhocoris apterus* L.). The fermentation products of glucose are acetic acid, lactic acid, ethanol, CO₂, and H₂. The guanine-plus-cytosine content of the deoxyribonucleic acid is 60 to 61 mol%. The biochemical and physiological features of this organism distinguish it from members of previously described genera. We propose a new genus, *Coriobacterium*, and a new species, *Coriobacterium glomerans*. The type strain is *C. glomerans* PW2 (= DSM 20642).

A distinct relationship exists between the intestinal symbionts and the nourishment of insects. The symbionts fulfill a vital task in digestion of food. They are transmitted to the offspring by oral uptake by the young brood or by smearing of the eggs (6, 14). Some heteropterous land bugs have adapted themselves to an extremely dry diet of ripe seeds. Red soldier bugs feed on dry seeds of lime trees and mallows.

We have studied the bacterial flora (5) of the intestines of red soldier bugs (*Pyrrhocoris apterus*; Heteroptera: Pyrrhocoridae). Only the third bulbous midgut portion of the intestine forms a habitat for bacteria. In aerobic cultures several species of *Streptococcus* and *Hafnia* were found (5). Under anaerobic conditions a gram-positive, heterofermentative bacterium was isolated (5). This organism was associated with all life stages of the bugs except the eggs. This bacterium is believed to be a symbiont of *P. apterus*, and it is considered to be a member of a new genus.

MATERIALS AND METHODS

Strains. Red soldier bugs were collected under dead foliage near the trunks of lime trees in five different places in Bavaria, Federal Republic of Germany (5); 89 strains of bacteria were isolated from these bugs. For detailed studies strain PW2^T (T = type strain) was used.

Microscopy. Gram-Weigert staining (2) and electron microscopy (8) were performed as described previously.

Media and growth conditions. The bacteria were enriched on blood agar plates (Columbia agar base [BBL, Div. Becton Dickinson and Co., Heidelberg, Federal Republic of Germany], 42.5 g/liter) containing defibrinated sheep blood (80 ml/liter) and on supplemented Schaedler agar plates (Schaedler agar [BBL], 41.9 g/liter) containing 50 ml of defibrinated sheep blood per liter and 15 mg of menadione (Sigma Chemical Co., Munich, Federal Republic of Germany) per liter. Incubation was carried out in a GasPak anaerobic system (BBL). The plates were incubated at 25 and 30°C for 10 to 20 days.

The organisms were further purified on blood agar, supplemented Schaedler agar, and Trypticase-Phytone-yeast extract (TPY) agar plates (11).

Some studies were made under strict anaerobic conditions in TPY medium (11) reduced with Na₂S (0.45 g/liter) and cysteine hydrochloride (0.45 g/liter) by using the culture

technique of Balch et al. (1). The gas phase in the serum bottles was N₂-CO₂ (80:20).

The ability of the isolate to ferment various carbohydrates (10 g/liter) was tested in TPY medium (11) lacking glucose but containing bromocresol purple (30 mg/liter). The carbohydrates were usually added before autoclaving. L-Arabinose, D-xylose, D-ribose, and lactose were added as filter-sterilized solutions. The incubation temperature was 30°C. Readings were made after 4, 8, and 14 days.

Cell wall analysis. The cell walls were analyzed as described previously (5).

G+C content. Deoxyribonucleic acid (DNA) was isolated as described by Wildgruber et al. (16). The guanine-plus-cytosine (G+C) content of the DNA was determined by using the methods of Zillig et al. (17) and Marmur and Doty (9).

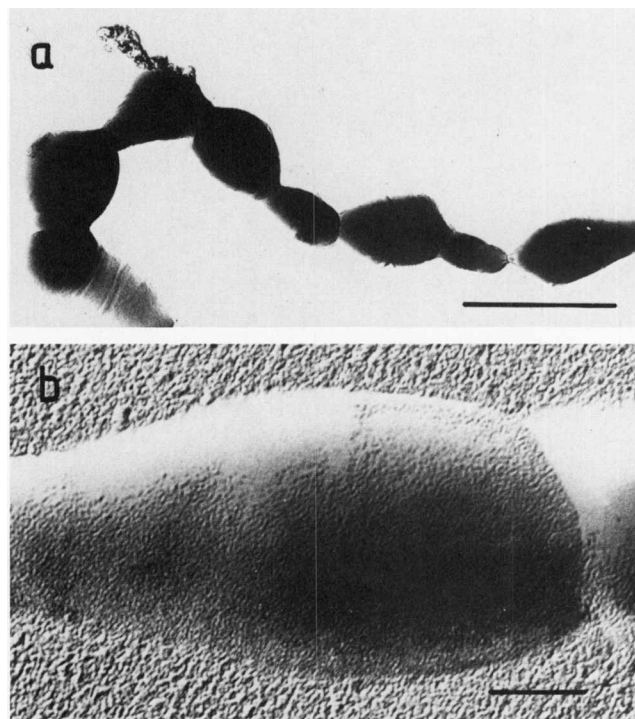


FIG. 1. Electron micrographs of platinum-shadowed cells of strain PW2^T. (a) Filamentous chain of cells. Bar = 2 µm. (b) Single cell of a chain.

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TABLE 1. Fermentation products of strain PW2^T

Fermentation product	Concn (μmol/ml)	
	Gas Pak anaerobic system	Method of Balch et al. ^a
Glucose (consumed)	7.80	5.61
L-Lactic acid (produced)	6.46	5.97
Acetic acid (produced)	7.50	7.20
Ethanol (produced)	6.13	1.37

^a See reference 1.

Enzymatic assays. The occurrence of fructose-6-phosphate phosphoketolase was determined as described by Scardovi (11); the occurrence of acetate, ethanol, glucose, and formate was determined as described by Bergmeyer (3); and the occurrence of D- and L-lactate was determined by using the method of Stetter and Kandler (15) without methylene blue and without diaphorase.

Gas-liquid chromatography. Determinations of alcohols and organic acids were carried out as described previously (8). The program for organic acids was as follows: 120°C, 2 min isothermal, then 5°C/min, 160°C isothermal.

RESULTS AND DISCUSSION

Habitat and isolation. Histological sections of the third bulbous portion of midguts showed bundles of gram-positive chain-forming bacteria attached to the epithelia of the intestines (5). The chains could reach more than 150 μm into the lumen of the gut. In the smears of adult and newly molted adult insects and larval stages which we examined the chain-forming bacteria (Fig. 1) were numerous. These bacteria were isolated from 89 of 92 specimens tested from all stages of the life cycle except the eggs. Therefore, the strains which we isolated are considered to be symbionts of *P. apterus*. These strains are placed in the new genus *Coriobacterium*, since they differ in some features from previously described genera. The systematic position of the genus *Coriobacterium* has to be determined.

Description of *Coriobacterium* gen. nov. *Coriobacterium* (Co.ri.o.bac.ter'i.um. Gr. fem. n. *koris*, bug; Gr. neut. n. *bakterion*, a small rod; M.L. neut. n. *Coriobacterium*, rodlet associated with bugs). The cells form chains of pear-shaped to irregularly shaped rods. The bacteria are obligately anaerobic. The cells stain gram positive. Spores and flagella are absent.

The fermentation products of glucose are acetic acid, L-lactic acid, ethanol (Table 1), CO₂, and H₂.

The peptidoglycan belongs to the Lys-Asp type (Table 2).

The bacteria occur in the intestinal tracts of bugs.

The G+C content of the DNA is about 61 mol%.

The type species is *Coriobacterium glomerans*.

Description of *Coriobacterium glomerans* sp. nov. *Coriobacterium glomerans* (glo'me.rans. L. part. adj. *glomerans*,

agglomerating; the cells form flocculent, wooly sediments with a clear supernatant in fluid media). The cells are pear-shaped to irregularly shaped rods. The bacteria are strictly anaerobic. The cells stain gram positive. The length of the cells varies from 0.44 to 1.80 μm. Spherical involution forms are common. The bacteria form chains more than 150 μm long. Flagella are absent.

The colonies consist of long filamentous chain-forming bacteria, bent into hairpins, curls, and loops. The colony size is about 0.6 mm. A flocculent, wooly sediment with a clear supernatant is formed in fluid media.

The organisms grow on blood agar (Columbia agar base [BBL]), supplemented Schaedler agar (BBL), and TPY agar at 25 and 30°C.

When grown in TPY medium, the bacteria produce from glucose acetic acid, L-lactic acid, and ethanol in a molar ratio of 1.16:1.00:0.95 (Table 1). CO₂ and H₂ also are produced (N. Weiss, personal communication). D-Lactic acid, formic acid, volatile short-chain alcohols, or other volatile fatty acids are not formed. Under more stringent anaerobic conditions (1) with N₂-CO₂ (80:20) as the gas phase, but lacking H₂, the molar ratio of acetic acid to L-lactic acid to ethanol is 1.20:1.00:0.23. This indicates that in the presence of the higher concentrations of molecular hydrogen produced by the commonly used GasPack anaerobic system strain PW2^T forms increased amounts of ethanol.

The cells ferment glucose, L-arabinose, D-xylose, D-ribose, mannose, sucrose, maltose, cellobiose, mannitol, and salicin. Lactose, melibiose, raffinose, inulin, starch, and inositol are not fermented.

The cells have an electron-dense gram-positive cell wall 40 nm wide (5). The peptidoglycan belongs to the Lys-Asp type (Table 2).

The bacteria occur in the third bulbous midgut portion of all stages of red soldier bugs (*P. apterus*) except the eggs. The filamentous cell chains are attached to the epithelia of the intestines.

The G+C content of the DNA is 60 mol%, as determined by quantitation of the 5' nucleotides after nuclease P1 treatment of the DNA (17), and 61 mol%, as determined by melting point analysis (9).

The type strain is *C. glomerans* PW2 (= DSM 20642).

Taxonomic comments. Strain PW2^T is distinguished from members of the genus *Lactobacillus* by its higher G+C content (strain PW2^T, 60 to 61 mol%; *Lactobacillus*, 32 to 53 mol% [7]) and by the molar ratio of the fermentation products (Table 1).

Strain PW2^T shares some characteristics with the genus *Bifidobacterium* (e.g., G+C content of the DNA [55 to 67 mol%] [11, 12]), and it possesses the type 6 murein of this genus (12). Based on its ability to ferment carbohydrates, it does not correspond to any species in this group (12). It differs from the genus *Bifidobacterium* by its lack of a positive test for fructose-6-phosphate phosphoketolase (11, 12) and by production of hydrogen. The high amount of

TABLE 2. Chemical composition of the cell walls of strain PW2^T

Treatment	Cell wall components (molar ratio)								
	Asp	Glu	Gly	Ala	Lys	NH ₃	Mur	GlcN	X ^a
4 N HCl	0.45	1.00	0.31	1.63	0.45	0.79	1.06	2.84	0.29
6 N HCl	0.91	1.00	0.45	1.96	1.01	ND ^b	ND	ND	ND

^a X, ε - (Aminosuccinyl)-lysine. For this calculation the extinction coefficient of lysine was used. ε - (aminosuccinyl)-lysine was also identified by thin-layer chromatography (13).

^b ND, Not determined.

ethanol produced cannot be explained by the hexose fermentation pathway in bifidobacteria.

Strain PW2^T is distinguished from *Eubacterium* by having a different DNA G+C content (*Eubacterium*, 30 to 55 mol% [10]), usually by its fermentation products (Table 1), and by its murein composition (Table 2). Several members of the genus *Eubacterium* produce hydrogen and acetic and lactic acids as the sole major acid products, but among these species, where the G+C content of the DNA is known, it is about 45 mol% (10); also, the species of *Eubacterium* which have been investigated possess the quite different type B murein (10, 13; F. Fiedler, personal communication).

The genus *Lachnospira* has been defined as containing curved to helical subterminally flagellated rods (4). Strain PW2^T is immotile, and flagella have not been observed by electron microscopy (Fig. 1). *Lachnospira* forms formic acid from glucose (4), while formic acid was not found among the fermentation products of strain PW2^T.

LITERATURE CITED

1. Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* **43**:260-296.
2. Berg, H. 1972. *Histologische Labortechnik*. J. F. Lehmanns, Munich.
3. Bergmeyer, H. U. 1974. *Methoden der enzymatischen Analyse*. Chemie, Weinheim.
4. Bryant, M. P. 1984. Genus *Lachnospira*. p. 661-662. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
5. Haas, F., and H. König. 1987. Characterization of an anaerobic symbiont and the associated aerobic bacterial flora of *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae). *FEMS Microbiol. Ecol.* **45**:99-106.
6. Henry, S. M. (ed.). 1967. *Symbiosis*, vol. 2. Academic Press, Inc., New York.
7. Kandler, O., and N. Weiss. 1986. Genus *Lactobacillus*, p. 1209-1234. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
8. König, H., and K. O. Stetter. 1982. Isolation and characterization of *Methanobolus tindarius*, sp. nov., a coccoid methanogen growing only on methanol and methylamines. *Zentralbl. Bakteriologie. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C* **3**: 478-490.
9. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
10. Moore, W. E. C., and L. V. Holdeman. 1986. Genus *Eubacterium*, p. 1353-1373. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
11. Scardovi, V. 1981. The genus *Bifidobacterium*, p. 1951-1961. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes*, vol. 2. Springer-Verlag, Berlin.
12. Scardovi, V. 1986. Genus *Bifidobacterium*. p. 1418-1434. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
13. Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407-477.
14. Steinhaus, E. A. 1967. *Insect microbiology*. Hafner Publishing Co., New York.
15. Stetter, K. O., and O. Kandler. 1973. Eine Schnellmethode zur quantitativen Bestimmung von L(+)- und D(-)-Milchsäure in Lebensmitteln. *Z. Lebensm. Unters. Forsch.* **152**:257-262.
16. Wildgruber, G., M. Thomm, H. König, K. Ober, T. Ricchiuto, and K. O. Stetter. 1982. *Methanoplanus limicola*, a plate-shaped methanogen representing a novel family, the *Methanoplanaceae*. *Arch. Microbiol.* **125**:259-269.
17. Zillig, W., K. O. Stetter, S. Wunderl, W. Schulz, H. Priess, and I. Scholz. 1980. The *Sulfolobus*-*Caldariella* group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. *Arch. Microbiol.* **125**:259-269.