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Composition and Biological Properties of Lipopolysaccharides Isolated from *Schizothrix calcicola* (Ag.) Gomont (Cyanobacteria)

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The most common cyanobacterium contaminating drinking water systems in southwestern Pennsylvania is *Schizothrix calcicola*. Lipopolysaccharides (LPS) were isolated from this species by hot phenol-water extraction. The polysaccharide moiety was composed of glucosamine, galactose, glucose, mannose, xylose, and rhamnose. The lipid A part contained beta-hydroxylauric, myristic, pentadecanoic, palmitic, beta-hydroxypalmitic, stearic, oleic, and linoleic acids. In contrast to many LPS isolated from *Enterobacteriaceae*, the dominant component was not beta-hydroxymyristic but beta-hydroxypalmitic acid. The LPS induced *Limulus* lysate gelation and Schwartzman reaction but was nontoxic to mice. The identity of LPS was verified by alkali and lysozyme treatment. The results suggest that *S. calcicola* is one of the principal sources of endotoxins in water systems using open finished-water reservoirs.

A water-borne outbreak of gastroenteritis affected approximately 5,000 persons in Sewickley, Pa., during August 1975. Extensive microbiological and chemical analyses of specimens obtained from patients and of water samples failed to identify a causative agent. However, investigation of the Sewickley water system revealed an accumulation of *Schizothrix calcicola* (*Cyanobacteria* = *Cyanophyta*) in the open finished-water reservoirs (15). Even 1 month after the outbreak, the water in the reservoir with the longest detention time was still contaminated by this species (400,000 cells ml^{-1}).

Toxic algae and cyanobacteria have been recognized since antiquity. They are found in diverse aquatic environments and have been implicated in poisoning and death of fish, wildlife, domestic animals, and humans (18). Many observed episodes were caused by ingestion of water from reservoirs, lakes, and ponds contamined by cyanobacteria. Since these organisms are widely distributed, it is not surprising that cyanobacteria were associated with several waterborne outbreaks of gastroenteritis in the United States (25, 26), the Philippines (3), and India (7). *S. calcicola* is not usually listed among the toxic cyanobacteria, and there is only one publication suggesting that this species may form toxic strains (1).

In addition to toxins (anatoxin, saxitoxin, toxic polypeptides), macromolecular substances characterized as endotoxins (lipopolysaccharides [LPS]) have been isolated from six species of cyanobacteria (2, 17, 29, 30). LPS are constituents of the outer cell wall of some microorganisms, predominantly gram-negative bacteria. These macromolecular substances are composed of three regions. The first region is the O-specific polysaccharide consisting of repeating oligosaccharide units, different in various species of bacteria. The second region is the basal core oligosaccharide, and the third region is the hydrophobic lipid part, the lipid A. Many gram-negative bacterial mutants possess only glycolipids, and the O-specific polysaccharide is absent. Sometimes even a part of the core oligosaccharide is lacking.

The LPS derived from blue-green organisms are generally identical to those occurring in gram-negative bacteria. However, the available though limited qualitative data on the composition of cyanobacterial LPS have already revealed a diversity in their chemical composition and biological characteristics.

In this report we describe the composition and biological properties of LPS isolated from *S. calcicola*.

MATERIALS AND METHODS

Enumeration of algae and cyanobacteria. Algae and cyanobacteria were counted in samples collected from five different water systems in the Pittsburgh, Pa., area. All of the water treatment plants selected for sampling stored finished water in open

reservoirs which varied in size from 0.5 to 177.5 million gallons. The samples were collected at weekly intervals during late spring and early summer (April–June 1977) from the treated water in each plant, open reservoir(s), and distribution system(s). Algae were preserved with Lugol solution, concentrated by sedimentation, and enumerated in a Palmer-Maloney nannoplankton cell. The results were expressed as number of cells per milliliter.

Cultures of *S. calcicola*. A brown strain of *S. calcicola* identical to that occurring in drinking water was isolated from Mosquito Creek Reservoir, near Youngstown, Ohio. It was identified by using cell morphology, color, and size and by comparison with the organisms obtained from Sewickley Reservoir no. 4. The identification was confirmed by Francis Drouet of the Academy of Natural Sciences of Philadelphia. *S. calcicola* was mass cultured in 9-liter bottles using Allen medium (9) kept at 24°C, aerated with compressed air at a rate of 100 to 250 ml/min, and illuminated by white fluorescent light adjusted to a 16-h cycle. Each bottle was inoculated with 200 ml of pure, aerated starter culture. For comparison, several mass cultures of *S. calcicola* were prepared in a 14-liter Microferm MF-114 fermentor (New Brunswick Scientific Corp.) with aeration and illumination and equipped with constant temperature controls. For endotoxin isolation, 14-day-old cultures were harvested at 3,450 rpm using a flow-through centrifuge (Gyrostester, DeLaval Separator Co.), washed three times, and lyophilized.

The heterotrophic bacterial contamination of *S. calcicola* was determined by plating serial dilutions (0.85% saline) of the cultures on Trypticase soy agar (BBL Microbiological Systems) in duplicate. The plates were incubated for 72 h at 20 and 35°C. Because the majority of the bacteria contaminating *S. calcicola* were from the family *Enterobacteriaceae*, a laboratory strain of *Salmonella typhimurium* was selected as the biomass indicator. The bacterial biomass was calculated utilizing a 24-h culture of this strain in soybean casein digest broth as a standard. A series of dilutions in physiological saline containing this organism was plated on Trypticase soy agar medium, and the number of colonies of *S. typhimurium* present in the culture was determined. One liter of the same culture was centrifuged, washed three times, and lyophilized, and the dry weight of bacteria was established. Based on this standard, the average count of heterotrophic bacteria contaminating *S. calcicola* cultures constituted only 0.04% of the total biomass.

Isolation and purification of LPS. The harvested cells were washed three times with sterile, distilled water. Each washing in a Sorvall (RC-2B) centrifuge lasted 25 min at 5,500 rpm and 4°C. The resultant sediment was uniformly suspended in distilled water and freeze-dried. The lyophilized, LPS-containing material was extracted twice for 30 min with 45% phenol solution at 68°C according to Westphal and Jann (31). The aqueous phase was dialyzed against distilled water until all traces of phenol were removed, and then it was centrifuged at 5,000 rpm for 15 min. The supernatant was freeze-dried.

It has been shown that crude LPS from cyanobacteria may contain glucan (17, 29). This compound was eliminated by enzymatic hydrolysis with a beta-glucosidase as recommended by Volk (27). Cellulase (2.5 mg ml^{-1}; Sigma Chemical Co.) was dissolved in water and centrifuged at 37,000 × *g* for 15 min, and the supernatant was passed through a 0.45-μm-pore size membrane filter (Millipore Corp.). The crude LPS was suspended at 5 mg ml^{-1} in 0.05 M citrate buffer (pH 4), and a final concentration of 0.25 mg of enzyme was added per 1 mg of LPS. After 10 h of treatment at room temperature, a second equal amount of cellulase was added, and the enzymatic hydrolysis was continued overnight. Afterwards the mixture was dialyzed for 48 h against tap water, concentrated in a rotary evaporator, and freeze-dried. To remove most of the nucleic acids, an aqueous solution (1 to 2%) of the freeze-dried, crude LPS was further purified by repeated (three times) centrifugation at 105,000 × *g* in a Spinco ultracentrifuge for 4 h. The sediment was resuspended in distilled water and centrifuged for 10 min at 3,000 rpm. The supernatant, representing the purified LPS, was lyophilized.

The phenol phase of the extracted cells was subjected to the same procedure as the aqueous phase, and only a negligible contamination by LPS was isolated from this fraction.

Analytical procedures. The neutral and amino sugars were released from the LPS by using hydrolysis with 1 N H_2SO_4 and 4 N HCl in a boiling-water bath for 4 and 12 h, respectively. After neutralization and subsequent centrifugation at 2,000 rpm for 10 min at 4°C, the supernatant was cooled and dried in a vacuum desiccator equipped with P_2O_5 and, in the case of amino sugars, also with NaOH pellets. Final analysis of the released sugars was performed using descending paper chromatography with proper sugar standards on Whatman no. 1 paper using *n*-butanol-pyridine-water (6:4:3, vol/vol/vol) (12).

For fatty acid analysis of the lipid A part, the LPS was hydrolyzed with 4 N HCl in a boiling-water bath for 5 h, followed by chloroform extraction repeated three times. The combined chloroform extract was dried in a vacuum rotary evaporator, and the residue was dissolved in ethyl ether and dried in a sand bath at 37°C. The sample was methylated with 0.5 ml of BF_3 methanol (14%, wt/vol) in a tightly stoppered flask, placed in a boiling-water bath for 2 min, and, after cooling, extracted twice with petroleum ether. The combined petroleum ether phases were dried at 60°C in a sand bath and dissolved in ethyl acetate before chromatographic and mass spectrometric analysis.

The methyl esters of the fatty acids were examined on a Packard series 7400 gas chromatograph (Packard Instrument Co., Downers Grove, Ill.) using a 3% OV-17 glass column (2 mm by 10 feet [ca. 3.05 m]). The analyses were programmed from 100 to 300°C at 7°C/min and held for 15 min with a chart speed of 0.25 inch (ca. 6.4 mm) per min. Helium was used as a carrier gas. The single peaks were further analyzed by using a LKB 900 mass spectrometer.

The following determinations were made by the respective methods: 2-keto-3-deoxyoctonate (KDO) by the thiobarbituric acid method; heptoses by the technique of Osborne; carbohydrates by the phenol-sulfuric acid method; proteins and total phosphorus

by the techniques of Lowry et al. All these chemical analyses were performed as microassays as described in the Keleti and Lederer handbook (12). In addition, the KDO was determined by the improved microassay technique described by Karkhanis et al. (10) using hydrolysis by 0.2 N H_2SO_4 at 100°C for 30 min.

Biological activity. The lethal effect of the purified endotoxin was tested in duplicate by intraperitoneal injections into five 20-g Swiss-Webster female mice for each concentration (100 μg, 500 μg, 1 mg, 2 mg, 4 mg).

Schwartzman phenomenon was performed on New Zealand rabbits using three different concentrations of LPS (80, 40, and 20 μg). The LPS in phosphate-buffered, pyrogen-free saline (pH 7.2) was injected subcutaneously into two adult rabbits. Sterile phosphate-buffered saline (pH 7) served as a control. Twenty-four hours later a challenge injection of LPS (40 μg) was given intravenously. The rabbits were observed 24 h later for signs of hemorrhage or necrosis.

LAL test. For the *Limulus* amoebocyte lysate (LAL) test, several serial solutions of LPS were prepared in pyrogen-free water (Travenol for injection [Travenol Lab. Inc.], divided into 100-ml portions and autoclaved for 1 h in 250-ml flasks). The assays were performed by adding 0.1 ml of the reconstituted lysate each to pyrogen-free tubes (10 by 75 mm), followed by the addition of 0.1 ml of LPS suspension. The tubes were thoroughly agitated by a Vortex mixer, covered with aluminum foil, and incubated for 60 min at 37°C in a water bath. A positive control containing 2.5 ng ml^{-1} of *Escherichia coli* endotoxin and a negative control (pyrogen-free water) were used in the assay. After the incubation period, the tubes were carefully inverted to determine presence of gelation.

Lysozyme and alkali treatment. The macromolecular compounds isolated from *S. calcicola* were tested for LPS content and possible occurrence of peptidoglycan by using lysozyme and alkali treatment. The isolated material was treated with three-times-crystallized egg white lysozyme (100 μg mg^{-1}) at pH 6.2 in 0.85% saline kept in a water bath at 37°C for 18 h (32).

Alkali treatment was accomplished using the technique described by Suzuki et al. (24). The material was suspended in pyrogen-free 0.1 N sodium hydroxide solution, which was kept in a sealed tube in a water bath at 70°C for 4 h. After neutralization with pyrogen-free hydrochloric acid, the samples were subjected to the LAL gelation test.

RESULTS

The field study results show the absence of living photosynthetic organisms (algae and cyanobacteria) in plant-finished water. However, substantial accumulations of these nuisance organisms were found in open finished-water reservoirs.

Green algae (*Chlorophyta*) are the most common and abundant nuisance organisms contaminating storage reservoirs and distribution systems during the summer. Cyanobacteria are quantitatively less important but may develop into a monospecific bloom if left unchecked (15). The most common cyanobacterium in open finished-water storage facilities in the Pittsburgh area (and probably in the entire United States) is *S. calcicola*. It was frequently found in three out of six reservoirs and one distribution system. However, the cellular concentrations were usually low, with high values recorded on few occasions (Table 1). Lesser quantities of algae and cyanobacteria were present in distribution systems, indicating contamination of drinking water during storage (Table 1). A portion of the photosynthetic organisms was destroyed by residual chlorine or by the permanent darkness or pressure in the mains. However, even in the adverse environment of distribution systems the living cells may persist for a prolonged period, because not all treatment plants postchlorinate the water leaving the reservoirs and entering the distribution system. The chlorine content was very low or at times absent in tap water, with means ranging from 0 to 0.6 mg of Cl per ml.

The LPS isolated from *S. calcicola* contained the neutral sugars glucose, galactose, mannose, xylose, and rhamnose, whereas the amino sugars were represented only by glucosamine (Table 2).

Both assays for KDO, including an improved microassay technique (10), were negative, and no color was obtained, indicating that this substance was not present in the LPS of *S. calcicola* (Table 2). The absence of KDO is also indicative of insignificant bacterial contamination, because this component is a well-known constituent of LPS of gram-negative bacteria. KDO was also absent in LPS derived from *Anabaena variabilis* (29). Buttke and Ingram (2) found only a negligible quantity (1.13% of LPS) of this compound in *Agmenellum quadruplicatum*, whereas LPS from *Synechococcus* (*Anacystis*) *nidulans* and *Phormidium* spp. contained from 0.5 to 1.5% (17, 30).

Similarly, as in the other cyanobacteria investigated for LPS content, heptose was absent in *S. calcicola* LPS. However, aminoheptose was discovered by Weise et al. in *Synechococcus* (*Anacystis*) *nidulans* LPS.

S. calcicola LPS contained 63% carbohydrates, whereas *Phormidium* spp., *Agmenellum quadruplicatum*, *Anabaena variabilis*, and *Synechococcus* (*Anacystis*) *nidulans* LPS contained 60, 59.5, 80.3, and 60%, respectively (2, 17, 29, 30).

The total phosphorus content in cyanobacterium LPS was found to be rather low, ranging from less than 1% to 3%, whereas in *S. calcicola* LPS it constituted less than 0.1%.

The LPS from *S. calcicola* was contaminated by 7.8% proteins. *Phormidium* spp., *Anabaena variabilis*, and *Agmenellum quadruplicatum* LPS contained 7 to 20, 8.4, and 0.13% proteins,

TABLE 1. *Distribution of algae and cyanobacteria in five drinking water systems located in Pittsburgh, Pa., area*

Locality	N^a	Total autrophic organisms (cells ml^{-1})		Total cyanobacteria (cells ml^{-1})		*Schizothrix calcicola*		
		Mean	Range	Mean	Range	Mean (cells ml^{-1})	Range (cells ml^{-1})	Frequency of occurrence (% of samples)
Dixmont Reservoir	10	77,400	2,400–216,600	160	0–1,500	160	0–1,500	20
Dixmont distribution	8	34,800	520–96,800	19	0–150	19	0–150	13
Highland Park I Reservoir	12	5,800	360–23,800	56	0–250	56	0–250	42
Highland Park (Pittsburgh) distribution	9	3,500	340–12,000	53	0–220	0	0	0
McKeesport Reservoir	12	2,100	110–6,500	470	0–2,100	460	0–2,100	67
McKeesport distribution	9	4,900	190–32,900	320	0–2,000	180	0–1,000	44
Sewickley no. 3 Reservoir	10	530	45–1,800	12	0–120	12	0–120	30
Sewickley no. 4 Reservoir	9	17,100	20–52,210	140	0–1,200	140	0–1,200	56
Sewickley low-pressure distribution	4	270	150–300	0	0	0	0	0
Sewickley high-pressure distribution	4	1,700	300–5,200	0	0	0	0	0
Springdale Reservoir	11	286,700	133,800–490,300	290	0–2,300	280	0–2,100	18
Springdale distribution	9	5,100	2,800–16,690	53	0–360	0	0	0

[a] *N*, Sample size.

TABLE 2. *Sugars, phosphorus, and proteins occurring in LPS of cyanobacteria*

Species	Sugars													KDO (%)	Total carbohydrates (%)	Phosphorus (%)	Protein (%)
	Glucose	Galactose	Mannose	Ribose	Arabinose	Xylose	Fucose	Rhamnose	3-*O*-Methylether-L-acofriose	Unidentified	Galactosamine	Glucosamine	Amino sugar ?				
Agmenellum quadruplicatum[a]	+	+	+	−	−	+	−	+	−	−	−	+	−	0.13	ND[b]	2.9	0.13
Anabaena variabilis[c]	+	+	+	−	−	−	−	+	+	−	−	+	−	0	80.3	0.03	8.4
Anacystis nidulans[d]	+	+	−	−	−	−	+	+	−	−	−	+	+	1.5	60	<1	ND
Phormidium spp.[e]	+	+	+	−	+	+	−	+	−	+	+	+	−	0.5–1.0	60	<1	7.20
Schizothrix calcicola	+	+	+	−	−	+	−	+	−	−	−	+	−	0	63	<0.1	7.8

[a] Data from Buttke and Ingram (2).
[b] ND, Not done.
[c] Data from Weckesser et al. (29).
[d] Data from Weise et al. (30).
[e] Data from Mikheyskaya et al. (17).
[f] ?, Unidentified.

respectively (2, 17, 29).

Gas-liquid chromatography and mass spectrometry identified eight long-chain fatty acids: beta-hydroxylauric, myristic, pentadecanoic, palmitic, beta-hydroxypalmitic, stearic, oleic, and linoleic. Stearic, oleic, and linoleic acids, containing 18 carbons each, were identified on the basis of differences in their molecular weights as detected by mass spectrometry and by comparison with proper standards. Surprisingly, the dominant component of lipid A was beta-hydroxypalmitic acid (Table 3), a constituent previously shown to be absent from several species of *Phormidium* (17), a genus closely related to *Schizothrix*. Several species of *Phormidium* are even thought to be ecophenes of *S. calcicola* (5).

The isolated and purified LPS was nontoxic to mice when injected intraperitoneally even at concentrations as high as 4,000 μg per mouse. Similarly, Weise et al. (30) have shown that LPS isolated from the cyanobacterium *Synechococcus* (*Anacystis*) *nidulans* was not toxic to mice. Mikheyskaya et al. (17) have demonstrated that glucan-free LPS from *Phormidium* spp. did not show any toxicity to mice either.

TABLE 3. *Fatty acids in LPS in cyanobacteria*

Fatty acid	*Agmenellum quadruplicatum*[a]	*Anabaena variabilis*[b]	*Anacystis nidulans*[c]	*Phormidium* spp.[d]	*Schizothrix calcicola*
Beta-hydroxylauric	−	−	−	−	+
Beta-hydroxymyristic	+	+	+	−	−
Beta-hydroxypalmitic	+?[e]	+	−	−	+
Beta-hydroxystearic	+?	+	−	−	−
Behenic	+	−	+	−	−
Lauric	−	−	+	−	−
Linoleic	−	−	−	−	+
Myristic	−	−	−	−	+
Oleic	−	−	+	+	+
Palmitic	+	+	+	+	+
Pentadecanoic	−	−	−	−	+
Stearic	−	−	+	+	+
Unidentified	−	+	−	−	−

[a] Data from Buttke and Ingram (2).
[b] Data from Weckesser et al. (29).
[c] Data from Weise et al. (30).
[d] Data from Mikheyskaya et al. (17).
[e] ?, Tentative identification.

Positive Schwartzman reaction using LPS isolated from *S. calcicola* was observed at concentrations of 40 and 80 μg. Signs of hemorrhaging and necrosis were observed on the sites of LPS injections 24 h later. Buttke and Ingram (2) recorded the same phenomenon when the LPS isolated from *Agmenellum quadruplicatum* was tested.

The result of the LAL reaction was positive for *S. calcicola* LPS (Table 4). The biological testing also included LAL assays with lysozyme- and alkali-treated LPS. The gelation almost disappeared after the treatment with alkali, whereas the lysozyme left the LPS intact. These results suggest that the purified phenol extract from *S. calcicola* contains true LPS and that the LAL gelation is not caused by peptidoglycans or other compounds which are also responsible for a positive LAL reaction (24, 33).

TABLE 4. *LAL gelation of LPS from S. calcicola*

LPS concn (μg ml^{-1})	Gelation[a]		
	No pretreatment	Lysozyme treatment	NaOH treatment
500	+++	+++	+
100	+++	+++	+
50	+++	ND	ND
10	++	++	−
1	+	++	−
0.5	+	+	−

[a] +++, Firm clot; ++, clot breaks; +, increase in viscosity; −, no reaction; ND, not done.

DISCUSSION

The planktonic and benthic strains of *S. calciola* have been found in a wide variety of environmental conditions, and this species was described as "perhaps the most widely distributed and common blue-green alga on earth" (5). It has been discovered in hot springs, in meltwaters of Antarctica, in the Dead Sea at an elevation of −1,286 feet (ca. −391.9 m), and in the Himalayas at an altitude of over 17,000 feet (ca. 5,182 m). It is not surprising that *S. calcicola* can withstand adverse conditions in open finished-water reservoirs and can form occasional water blooms in drinking-water systems. Therefore, the possible health effect of LPS derived from this species is worthy of investigation.

The yield of LPS isolated from *S. calcicola* as compared to other cyanobacteria (17, 29, 30) was unusually high (7%). This suggested contamination of the LPS by glucan, a phenomenon noted by several authors (6, 17, 29). An electron microscopy study of cyanobacteria detected mucous cellular envelopes containing glucan (6). Weckesser et al. (29) found the same substance in the water phase of a phenol-water extract from *Anabaena variabilis*, whereas LPS was isolated only from the phenol phase. In addition, glucans were also discovered in crude LPS isolated from the cell walls of the genus *Phormidium* (17). After the removal of glucan, the average yield of purified LPS from whole *S. calcicola* cells decreased to 1.5 to 2%, which is similar to gram-negative bacteria.

The chemical analysis of LPS from *S. calcicola* revealed that KDO was not present in this macromolecular substance (Table 2). However, this component was absent in endotoxins isolated from *Anabaena variabilis* (29) and was found in small or negligible quantities in endotoxins of three other species [*Agmenellum quadruplicatum*, *Synechococcus* (*Anacystis*) *nidu-*

lans, *Phormidium* spp.] (2, 17, 30). This compound is lacking even in some gram-negative bacteria such as *Bordetella* (16). Therefore, KDO is not necessarily an essential component of the core part of LPS.

Another important aspect is the possible use of the chemical composition of LPS for taxonomical classification of cyanobacteria. In the past, similar characterization has been performed on *Salmonella* and *Escherichia coli*, when serotypes were classified according to the sugar composition of their LPS into 40 groups called chemotypes (11, 19). Similarly, the *Citrobacter* serotypes were divided into 20 chemotypes by Keleti et al. (13, 14) and Sedlak et al. (21, 22).

Until recently the taxonomy of cyanobacteria was based mostly on cell morphology and size. Relatively very few comparative taxonomic studies on physiological and chemical properties of cyanobacterial cells were published (23). It is, therefore, possible that the chemical characterization of cyanobacterial LPS or other cell wall components may shed more light on the taxonomy of cyanobacteria.

There is some evidence that not all the species of cyanobacteria contain LPS. Wang and Hill (28) believe that the occurrence of LPS among cyanobacteria cannot be generalized. They isolated only a polysaccharide from *Anabaena flos-aquae*, while the lipid A part was completely absent. However, Weckesser et al. (29) isolated a true LPS from another species of the same genus (*Anabaena variabilis*). Such an interspecific difference in biochemical composition of procaryotic cells is rather surprising, especially when considering the yield of the fatty acid material obtained by Weckesser et al. (29) from acid hydrolysates by extraction with petroleum ether (10.7% of the LPS dry weight). Therefore, it is important to examine all the species of cyanobacteria commonly occurring in water systems for LPS content.

The isolated endotoxin from *S. calcicola* was nontoxic to mice when injected intraperitoneally but caused LAL gelation and positive Schwartzman reaction. The low toxicity of LPS to mice cannot be considered a decisive factor in determining the health effects of this substance to humans. All LPS isolated from six species of cyanobacteria have been found to be nontoxic to mice. Even LPS isolated from *Yersinia pestis* exert very low toxicity, and 5 to 10 mg of this endotoxin is required to kill a mouse. Endotoxin isolated from R-mutants of gram-negative bacteria have also much lower toxicity to mice than LPS derived from wild types (16).

All types of bacterial LPS may cause endotoxemia, especially in debilitated, immunosuppressed patients and infants. Enhanced gastrointestinal permeability in infants suggests that absorption of exogenously derived endotoxin (from milk or water) could initiate illness under certain conditions. For instance, interaction of lead or some other agents present in the environment (i.e., water) with endotoxin may result in harmful effects upon oral ingestion (4). Interestingly enough, the highest value (800 ng ml^{-1}) of LPS was detected by DiLuzio and Friedman (4) in the tap water of Mexico City. This high endotoxin level does not seem to affect the indigenous population but may cause "traveler's diarrhea" in tourists.

The presence of endotoxins in drinking water and the effect of LPS introduced into the bloodstream have been observed by Hindman et al. (8) while studying an epidemic of pyrogenic reactions among kidney dialysis patients. This epidemic occurred at the same time that maximum concentrations of algae and cyanobacteria were observed in the raw water.

The oral ingestion of endotoxins produced by cyanobacterial blooms in the treated water stored in uncovered reservoirs may be responsible for some of the water-borne disease outbreaks presently classified as "of unknown etiology." For example, the presence of high concentrations of endotoxins (2,500 ng ml^{-1}) in the Sewickley, Pa., water system measured by the Environmental Protection Agency shortly after the outbreak of water-borne gastroenteritis of unknown origin may lead to a speculation on a possible relationship between the consumption of LPS derived from *S. calcicola* (400,000 cells ml^{-1}) and the reported illness. Under normal operating conditions during the spring and summer of 1977, concentrations of *S. calcicola* in this reservoir were much lower, ranging from 0 to 1,200 cells ml^{-1}, and the corresponding LPS concentrations were between 25 and 250 ng ml^{-1}.

Peptidoglycans of gram-positive bacteria were described as having endotoxin-like properties as well but at much higher concentrations than LPS (20). Even though Stanier and Cohen-Bazire (23) have shown that cyanobacterial cells contain a peptidoglycan layer which varies in width with different species, our data indicate that this constituent probably occurs in *S. calcicola* in small quantities and does not contribute substantially to LAL reaction. Our recent work has shown that even a thorough treatment of *S. calcicola* cells by lysozyme did not decrease the LAL gelation activity (lysozyme breaks down the peptidoglycans into inactive compounds).

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