**Note**

**Cellulose as Extracellular Polysaccharide of Hot Spring Sulfur-turf Bacterial Mat**

Kazutoshi OGAWA¹ and Yonosuke MAKI²,†

¹Department of Environmental Science, College of Science and Engineering, Iwaki Meisei University, Iwaki 970-8551, Japan

²Unit of Environmental Science, Faculty of Humanities and Social Sciences, Iwate University, Morioka 020-8550, Japan

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The carbohydrate fraction of a hot spring sulfur-turf bacterial mat was shown to contain cellulose by the examination of neutral sugar composition, methylation analysis, and the identification of free oligosaccharides obtained from an acetolyzate of the desulfurized sulfur-turf mat. This suggested that the sulfur-oxidizing bacteria composing the sulfur-turf were producers of cellulose.

**Key words:** cellulose; extracellular polysaccharide; sulfur-turf; sulfur-oxidizing bacteria

Sulfur-turf is a massive aggregate of sulfur-oxidizing bacteria growing in hot spring effluents containing some dissolved sulfides, and has a turf-like appearance because of the adherence of many elemental sulfur particles.¹,²) Microscopic observation revealed that the large sausage-shaped bacteria that were dominant bacteria of the turf held sulfur particles in the extracellular polysaccharide.³) Microbiological characteristics of the bacterial cells of the sulfur-turf have been investigated, but no mention was made of the gelatinous extracellular polysaccharide. For this reason, we set out to study the chemical structure of the carbohydrate fraction of the sulfur-turf.

Hot spring sulfur-turf was collected from Ganiba Spa in Akita Prefecture and air-dried, and then crushed in a porcelain mortar (yield, 12.0 g). The crushed dry material was desulfurized with 120 ml of carbon disulfide, twice (yield, 4.2 g) and analyzed.

The desulfurized sulfur-turf (100 mg) was methylated twice by Hakomori’s method,⁶ then hydrolyzed, reduced, and acetylated with the method of Lindberg.⁴) The partially methylated alditol acetates thus obtained were analyzed by GLC using a Shimadzu CBP5 capillary column (0.2 mm ID × 50 m) at 200°C. The main peak was identified as 1,4,5-tri-O-acetyl-(1-deuterio)-2,3,6-tri-O-methylglucitol, showing the presence of 4-O-glycosylated glucose by standards prepared from glucobiose (retention times relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol of tri-O-acetyl-3,4,6-, 2,4,6-, 2,3,6, and 2,3,4-O-methylglucitol were 1.308, 1.320, 1.373, and 1.463, respectively), and this was confirmed by GC-MS analysis on a Jeol JMS-700 mass spectrometer (EI mode, 70 eV)⁷ as shown in Fig. 1. Therefore, it is clear that the carbohydrate fraction of the desulfurized sulfur-turf contains a 1,4-glucan.

The desulfurized sulfur-turf (15 mg × 4) was methylated twice by Hakomori’s method,⁶ then hydrolyzed, reduced, and acetylated with the method of Lindberg.⁴) The partially methylated alditol acetates thus obtained were analyzed by GLC using a glass column (0.3 cm ID × 175 cm) packed with 3% ECNSS-M on Chromosorb W (80–100 mesh) at 200°C. The results showed the carbohydrate fraction of desulfurized sulfur-turf contained glucose alone. The content of glucose measured by the method of Nelson-Somogyi⁵) was 45% with glucose as a standard.

The desulfurized sulfur-turf (15 mg × 4) was hydrolyzed with 120 ml of carbon disulfide, twice (yield, 4.2 g), and then evaporated to a syrup. This procedure was repeated six times and 381 mg of corresponding alditol acetate,⁴) and then its neutral sugar composition was examined by GLC using a glass column (0.3 cm ID × 175 cm) packed with 3% ECNSS-M on Chromosorb W (80–100 mesh) at 200°C. The results showed the carbohydrate fraction of desulfurized sulfur-turf contained glucose alone. The content of glucose measured by the method of Nelson-Somogyi⁵) was 45% with glucose as a standard.

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The desulfurized sulfur-turf (100 mg) was acetylated with stirring in a mixture of 4.5 ml of acetic acid, 4.5 ml of acetic anhydride, and 0.45 ml of conc. sulfuric acid for 3 d at room temperature. The mixture was poured into 25 g of ice and water, neutralized with sodium hydrogen carbonate (pH 4.5), and extracted with 50 ml of chloroform, twice. The extract was successively washed with 50 ml of water, 1 M hydrochloric acid, 1 M sodium hydrogen carbonate solution, and water, then dried under sodium sulfate, and evaporated to a syrup. This procedure was repeated six times and 381 mg of

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¹ To whom correspondence should be addressed. Fax: +81-019-621-6829; E-mail: maki@iwate-u.ac.jp
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syrups were obtained. Deacetylation of the acetol- 
yzate was done in the usual procedure with 0.15 M methanolic sodium methoxide (yields: 243 mg). The 
residue was fractionated by gel filtration (15 mm × 
900 mm column) on Bio-gel P-2 (fine, 45–90 μm, Bio- 
Rad Laboratories, California), eluting with water at 
40°C. A fraction of 40 drops (1.3 ml) was collected. 
The eluate was divided into three fractions, tube Nos. 
67–69 (tetrasaccharide fraction) (1), Nos. 73–75 (tri- 
(2), and Nos. 79–85 (di-) (3). The concentration of 
each fraction yielded 6.7, 17.8, and 38.7 mg of solids, 
respectively. 1H-NMR spectra of these fractions were 
recorded at 400 MHz with a Jeol JNM-ECP400 spec- 
trometer on solutions in deuterium oxide (internal 
acetone for 1H, 2.217 ppm), and compared with 
those of authentic malto- and cello-oligosaccharides 
purchased from Sigma Chemical Co. (St. Louis, 
MO). These results showed that the fraction (1), (2), 
and (3) contained cellotetraose, cellotriose (see 
Fig. 2), and cellbiose, respectively. Optical rotation 
values taken on a Jasco DIP-370 polarimeter were all 
positive (α-series) as follows: (1), [α]D 24° (c 0.89, 
H2O) (lit.9) [α]D + 32.8 → + 21.0° 
(H2O); (3), [α]D 21° (c 1.9, H2O) (lit.10) [α]D 
+ 13 → + 34° (H2O)). Therefore, It was proved that 
a 1,4-glucan of the carbohydrate fraction of the 
desulfurized sulfur-turf was cellulose. 

This showed that the gelatinous extracellular 
polysaccharides of the sulfur-turf contained cellu- 
llose, and suggested the sausage-shaped sulfur- 
oxidizing bacteria, main composers of the turf, were 
producers of cellulose. Cellulose production has 
been established not only for Acetobacter xylinum 
(Gluconacetobacter xylinus)11) and other gram-nega- 
tive bacteria, but also for gram-positives.12) Although 
several species of phototrophic cyanobacteria were 
reported as cellulose producers,13) it has not been 
known that a chemolithotrophic bacterium such as a 
sulfur-oxidizer synthesizes cellulose. It was reported 
that the sausage-shaped bacterium formed a major 
cluster with members of the Aquifex-Hydrogenobac- 
ter complex,14) which is the most deeply branching 
bacterial group on a phylogenetic tree based on 16S 
rRNA gene sequences. Accordingly, there are very 
exciting perspectives not only for the biochemical
interests and its economical potentiality but also for phylogeny of domain bacteria. It is necessary to continue studying this subject.

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References