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

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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 acetylate 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 first hydrolyzed with 0.5 ml of 25 N sulfuric acid for 5 h at 25°C, then we diluted the reaction material with 12 ml of water and heated it for 11 h in a boiling water-bath. The reaction mixture was neutralized with barium carbonate, and the filtrate was diluted into 100 ml with water. A portion of the acid hydrolyzate was deionized with Dowex 50W resin (H+ form), evaporated to a syrup, and converted into the 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.

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.7) 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-deutero)-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-tri-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) 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 (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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syrups were obtained. Deacetylation of the acetolyzate 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 \times 900 mm column) on Bio-gel P-2 (fine, 45–90 \mu m, 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. \(^\text{1}^H\)-NMR spectra of these fractions were recorded at 400 MHz with a Jeol JNM-ECP400 spectrometer on solutions in deuterium oxide (internal acetone for \(^\text{1}^H\), 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 cellobetaose, cellotriose (see Fig. 2), and cellulobiose, respectively. Optical rotation values taken on a Jasco DIP-370 polarimeter were all positive (p-series) as follows: (1), \([\alpha]\)\(_D^0\) + 26° (c 0.33, H\(_2\)O) (lit.\(^8\) \([\alpha]\)\(_D^0\) + 7.1 → +17.1° (H\(_2\)O)); (2), \([\alpha]\)\(_D^0\) + 24° (c 0.89, H\(_2\)O) (lit.\(^9\) \([\alpha]\)\(_D^0\) + 32.8 → +21.0° (H\(_2\)O)); (3), \([\alpha]\)\(_D^0\) + 21° (c 1.9, H\(_2\)O) (lit.\(^10\) \([\alpha]\)\(_D^0\) + 13 → +34° (H\(_2\)O)). 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 cellulose, 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* (*Glucanacetobacter xylinus*)\(^11\) and other gram-negative bacteria, but also for gram-positives.\(^12\) Although several species of phototropic 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-Hydrogenobacter* complex,\(^14\) which is the most deeply branching bacterial group on a phylogenetic tree based on 16S rRNA gene sequences. Accordingly, they are very exciting perspectives not only for the biochemical

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**Fig. 1.** Gas Chromatogram of Partially Methylated Alditol Acetate from Permethylated Desulfurized Sulfur-turf (A) and Mass Spectrum and Mass Spectral Fragmentation Pattern of Main Peak in (A) (B).
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


