# 3-Amino-3-deoxy-D-glucose: an antibiotic produced by a deep-sea bacterium

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*Summary*. Gram-positive bacteria isolated from deep-sea sediments of the Pacific basin showed considerable antibacterial activity. A *Bacillus* strain, isolated from a sediment sample collected at a depth of 4310 m, was shown to produce 3-amino-3-deoxy-D-glucose, a known antibiotic.

*Key words*. Deep-sea bacteria; gram-positive; antimicrobial activity; *Bacillus;* 3-amino-3-deoxy-D-glucose.

Antimicrobial activities have been known for some time among bacteria and actinomycetes isolated from marine environments. Rosenfeld and Zobell[1] first showed antibiosis of marine bacteria nearly 40 years ago. Thereafter, many reports on antibacterial and antifungal properties of marine isolates of microorganisms have appeared[2–7]. In spite of the increasing knowledge in this field, only a few substances of an antibiotic nature have been isolated and characterized from marine microbes[8,9].

Hitherto, research efforts have been focused on coastal waters and sediments, where organic substrates are abundant. Although there has been a considerable number of reports of the isolation of bacteria from deep seas[10–12] sometimes as deep as 10000 m, little knowledge of their antimicrobial activities has accumulated. It is therefore understandable that no antimicrobial secondary metabolites have been reported from deep-sea microorganisms. Recently, we have successfully isolated more than 90 strains of deep-sea bacteria and examined their antimicrobial activities. Subsequently, we were able to isolate and identify an antibacterial substance from an isolate which was found in a sediment sample obtained from the Pacific basin at a depth of 4310 m. We describe these results here.

Deep-sea bacteria were isolated from the Pacific basin during the cruises KH-79-4 and KH-80-1 by the R/V HAKUHO-MARU of the Ocean Research Institute, University of Tokyo. Sediment samples were collected by an Ocean Grab or a box corer similar to that used by Hasseler and Jumars[13]. After lifting samples on board, cores were obtained by inserting a few cleaned plastic tubes (37 mm in diameter) into the sediments collected. Bottom deposits in the tubes were excluded, and 1-ml samples of wet sediment from the different core depths were removed with a sterile spatula and transferred into a glass tube containing 9 ml of sterilized seawater. The suspension was filtered through a Nucleopore filter (47 mm in diameter; 2.2-µm pore size). The filter was then placed on a PPES-II agar plate[14], which was incubated at 20 °C for 2 weeks. Colonies which appeared on the filter were counted and isolated. Mainly gram-positive bacteria were obtained, morphologically classified as *Bacillus* spp. and *Corynebacterium* spp.

Of 92 gram-positive isolates, we could maintain 88 strains on PPES-II agar plates. They were examined for antimicrobial activities against 8 microorganisms after being cultured under various conditions; 12 different culture media, temperatures, culture times, and shaking rates. Most of the isolates exhibited some antibacterial activity. The results with the six most active isolates are depicted in the table.

For isolation and characterization of antimicrobial compounds produced by these deep-sea bacteria, we first chose strain 5-15-3, which was isolated from the 15-cm core strata of sediments collected at a depth of 4310 m in the Izu Trench (26°17.5' N, 142°55.2' E). This isolate was gram-variable, facultatively anaerobic, and motile with lophotrichous flagella. It was a catalase-positive, small rod (0.5–0.8 × 1.2–2.0 µm) (fig. 1) occurring as single cells. An endospore was formed in the center or the lateral position in the cell (fig. 2). The bacterium grew well at temperatures between 15 and 50 °C. It was negative to Voges-Proskauer, methyl-red and nitrate reduction tests; hydrolyzed starch grew under anaerobic condition and accumulated no poly-ß-hydroxybutyrate. Trehalose, fructose, glucose, L-arabinose, D-xylose, sucrose, inositol, D-galactose, D-sorbitol, and salicin were utilized by the bacterium as a sole carbon source. These features indicated that this isolate may represent a new species of the genus *Bacillus*.

The isolation of an antibacterial compound was done as described below. The bacterial isolate was cultured at 20 °C for 48–72 h with aeration and agitation in 500-ml Erlenmeyer flasks containing 50 ml of a culture medium which consisted of glycerol (3%), glucose (0.1%), sodium thiosulfate (0.1%), peptone (0.5%), meat extracts (0.5%) and sodium chloride (0.5%), and adjusted to pH 7.0. The broth (1 l) was adjusted to pH 2.0 and applied to a Dowex 50W-X8 ($NH_4^+$ form) column. The column was washed thoroughly with water followed by 0.1 M aqueous ammonia. The latter eluate which contained antibacterial activity was freed from ammonia and chromatographed over Dowex 1-X8 ($AcO^-$ form) with water. Antibacterial fractions were subjected to column chromatography on AG 50W-X8 ($NH_4^+$ form) with a linear gradient from water to 0.1 M aqueous ammonia. Fractions which gave a yellowish purple spot at Rf 0.40 on Kieselgel $GF_{254}$ plates when developed with a mixture of 2-propanol, methanol and 17% aqueous ammonia (1:2:1) and visualized with ninhydrin reagent, were combined, and finally purified

Antimicrobial activity of 6 bacterial isolates against 8 microorganisms. Strain 5-15-3 was cultured in medium A (glycerol 3.0%, glucose 0.1%, sodium thiosulfate 0.1%, peptone 0.5%, meat extracts 0.5%, sodium chloride 0.5%; pH 7.0) at 20 °C; 9-15-14 in medium A at 27 °C; Eq-3 in medium B (glucose 0.1%, yeast extracts 0.1%, polypeptone 1.0%, beef extracts 0.5%, sodium chloride 0.3%; pH 7.2) at 27 °C; Eq-11 in medium C (soybean meal 5.0%, sucrose 1.2%, ammonium sulfate 0.2%, calcium carbonate 0.2%; pH 7.0); Eq-15 in medium A at 30 °C; Eq-22 in medium C at 27 °C. Culture media were tested for antimicrobial activity by the paper-disc assay against *Staphylococcus aureus* 2217, *Bacillus subtilis* ATCC 6633, *Escherichia coli* K-12, *Proteus mirabilis* IFM OM-9, *Serratia marcescens* IID 620, *Pseudomonas aeruginosa* NCTC 10490, *Mycobacterium* sp. 607, and *Candida albicans* ATCC 10234. Activity is expressed by diameter (mm) of inhibitory zone.

| Strain | Collection site | Depth of collection | *S. aureus* | *B. subtilis* | *E. coli* | *P. mirabilis* | *Ser. marcescens* | *Ps. aeruginosa* | *Myc.* sp. | *C. albicans* |
|---|---|---|---|---|---|---|---|---|---|---|
| 5-15-3 | Izu Trench | 4310 m | 23.0 | 11.0 | 11.0 | 12.0 | 0 | 0 | 0 | 0 |
| 9-15-14 | Izu Trench | 8260 | 0 | 19.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Eq-3 | Equatorial Pacific | 2220 | 19.0 | 12.2 | 22.2 | 0 | 0 | 0 | 0 | 0 |
| Eq-11 | Equatorial Pacific | 2220 | 20.4 | 14.4 | (15.5)* | (13.0)* | 0 | 0 | (19.0)* | 0 |
| Eq-12 | Equatorial Pacific | 2220 | 0 | 0 | 0 | 16.2 | 0 | 0 | 0 | 0 |
| Eq-15 | Equatorial Pacific | 2220 | 0 | 20.0 | +** | 0 | 0 | 0 | 0 | 0 |

* Half inhibition. ** Weak inhibition.

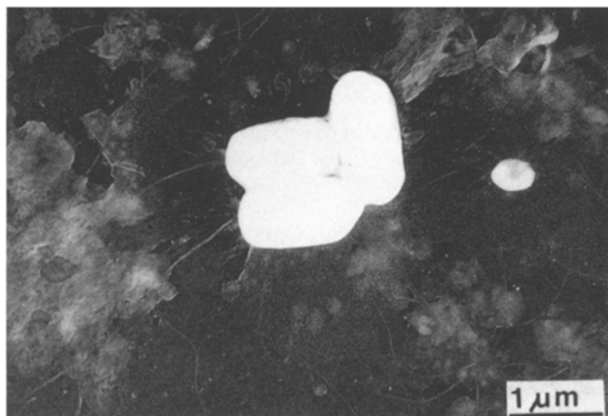


Figure 1. Electron microscope photograph of strain 5-15-3, ×15 000.

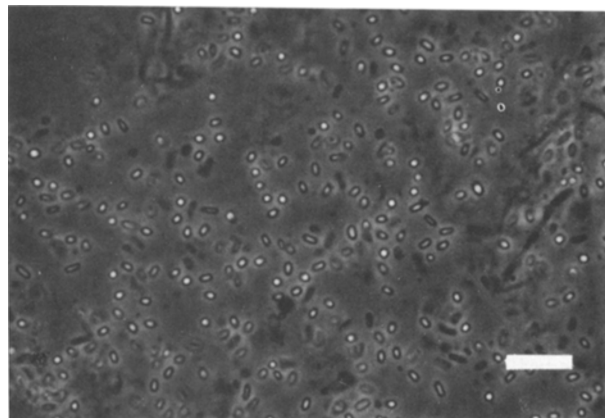

Figure 2. Endospores of strain 5-15-3 observed by using a microscope with Nomarski optics. Bar indicates 10 μm.

on CM-Sephadex C-25 ($NH_4^+$ form) with water-0.05 M aqueous ammonia gradient (1:1). Lyophilization of the active fractions afforded 200 mg of a white hygroscopic powder, $[\alpha]_D$ +53.5° (24 h after being dissolved in water, 10 mg/ml).

The antibiotic was labile as the free base, but stable as the hydrochloride or sulfate form. It was positive to ninhydrin and sugar reactions. The fast atom bombardment (FAB) mass spectrum showed an $(M + H)^+$ ion peak at $m/z$ 180, suggesting a monoamino sugar feature of the compound. Because of extreme hygroscopicity of the antibiotic we carried out the structural elucidation after conversion to the peracetate[15]. The combustion analysis [C, 49.3; H, 5.65; N, 3.53%, calculated for $C_{16}H_{23}O_{10}N$ (C, 49.3; H, 5.95; N, 3.59%)] and FAB mass spectrum [$m/z$ 390 $(M + H)^+$] of the peracetate led to a molecular formula of $C_6H_{13}O_5N$ for the antibiotic. The 400 MHz $^1H$ NMR analyses including extensive double resonance experiments, together with other spectral data[16], allowed us to assign the structure of the antibiotic as 3-amino-3-deoxy-glucose. In order to determine the absolute configuration, we prepared $\alpha$- and $\beta$-anomers of methyl 2,4,6-tri-*O*-acetyl-3-N-acetamidoglucoside from the antibiotic[17]. The difference of molecular rotations of $\alpha$- and $\beta$-anomers ($[M]_\alpha - [M]_\beta$) was +417.4°, indicating the D-configuration of the sugar[18]. Thus, the antibiotic isolated from the deep-sea bacterium was unambiguously identified as 3-amino-3-deoxy-D-glucose[19].

3-Amino-3-deoxy-D-glucose was first discovered from *Bacillus aminoglucosidicus* in 1967[20] and later from *Streptomyces lansus*[21]. It is also contained in such known antibiotics as kanamycins, 3-trehalosamine and hikizimycin. This amino sugar inhibits bacterial cell-wall synthesis[22]. Our isolation of 3-amino-3-deoxy-D-glucose from a deep-sea bacterium may be a clue suggesting that bacteria in deep-sea sediments are terrigenous in origin.

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16 IR spectrum (film): 3300, 1750, 1665, 1540, 1230, and 1060 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 1.90–2.20 ppm (s, $OCOCH_3$), 3.90 (ddd, J = 10.4, 4.4, 2.0 Hz, H-5$\beta$), 4.17 (ddd, 11.1, 3.6, 2.1, H-5$\alpha$), 4.10 (dd, 12.6, 2.0, H-6$\beta$a), 4.08 (dd, 12.3, 2.1, H-6$\alpha$a), 4.33 (dt, 9.6, 10.0, H-3$\beta$), 4.76 (dt, 11.2, 10.6, H-3$\alpha$), 4.35 (dd, 12.6, 4.4, H-6$\beta$b), 4.33 (dd, 12.3, 4.0, H-6$\alpha$b), 4.91 (dd, 8.4, 10.0, H-2$\beta$), 4.91 (dd, 10.0, 10.4, H-4$\beta$), 4.94 (dd, 10.6, 11.1, H-4$\alpha$), 5.00 (dd, 3.6, 11.2, H-2$\alpha$), 5.61 (brd, 9.6, NH-$\beta$), 5.54 (brd, 10.6, NH-$\alpha$), 5.76 (d, 8.4, H-1$\beta$), 6.26 (d, 3.6, H-1$\alpha$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.5 ppm (q, $OCOCH_3$, 8C), 22.8 (q, $NCOCH_3$, 2C), 49.3 (d, C-3$\alpha$), 53.2 (d, C-3$\beta$), 61.6 (t, C-6$\alpha$ and $\beta$), 67.9 (d, C-4$\alpha$)*, 68.1 (d, C-4$\beta$)*, 68.9 (d, C-2$\alpha$)*, 70.1 (d, C-2$\beta$)*, 70.4 (d, C-5$\alpha$), 73.4 (d, C-5$\beta$), 88.7 (d, C-1$\alpha$), 91.9 (d, C-1$\beta$), 168.7 (s, $NCOCH_3$, 2C), 170.2 (s, $OCOCH_3$, 8C). *Assignments may be interchanged.
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