MICROBIAL PRODUCTION OF 4-THIOURIDINE

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(Received for publication March 7, 1992)

A strain of *Streptomyces libani* NK-110134 (FERM 10638) has been found to produce 4-thiouridine (Fig. 1), which exhibits inhibitory activity against *Bacillus subtilis* and causes complete regression of HeLa S_3 cells. Chemical synthesis of 4-thiouridine was reported in 1959[1] and microbial ribosylation of 4-thiouracil in 1977[2]. The nucleoside is present as a minor component of tRNA in *Escherichia coli*[3].

We are reporting an efficient method for microbial production of 4-thiouridine as an alternative to chemical synthesis.

A loopful of a 10-day slant culture of *S. libani* NK-110134 grown on yeast extract - starch agar was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of a seed medium composed of soluble starch 2.0%, glucose 0.5%, peptone 0.5%, yeast extract 0.5%, soybean meal 0.5%, K_2HPO_4 0.05%, $MgSO_4$ 0.05% and $CaCO_3$ 0.2% (pH 7.2) prepared in tap water. After a 2-day incubation on a rotary shaker (190 rpm, 27°C), 2 ml of the seed culture were inoculated into 110 ml of the fermentation medium contained in 500 ml flasks. This medium consisted of glycerol 0.5%, yeast extract 0.3%, meat extract 0.5%, NaCl 0.3% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 7.0) prepared in tap water, and was employed as described above. The pH adjustments made in preparation of these media were carried out before sterilization for 15 minutes at 110°C.

The production of 4-thiouridine was determined by assaying the filtered fermentation beer daily for its activity against *B. subtilis*. After a 3-day incubation, the beer (10 liters) was filtered and the filtrate (pH 6.3) was applied to a Diaion HP-20 column (800 ml). The column was washed with 5 liters of water and was eluted with aqueous Me_2CO. The eluate obtained with 50% aqueous Me_2CO was evaporated to dryness. A MeOH-extract of the residue was prepared and evaporated to give a crude powder (5.5 g). This material was dissolved and chromatographed on a silica gel column (800 ml) using a mixture of $CHCl_3$ and MeOH (6 : 1). The active fractions were pooled and concentrated to give 130 mg of a yellowish oil. The oil was dissolved in MeOH and was applied to a Sephadex LH-20 column (900 ml). The column was eluted with MeOH and the active fractions were concentrated to give 50 mg of a yellow powder. Successive purification of this material using silica gel TLC with a solvent mixture of $CHCl_3$ - MeOH (6 : 1) gave 25 mg of pure yellow material with an overall yield of 2.5 μg/ml of

Fig. 1. Structure of 4-thiouridine.

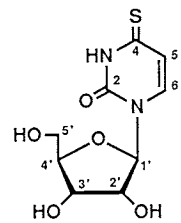


Table 1. The ^{13}C chemical shifts of 4-thiouridine.

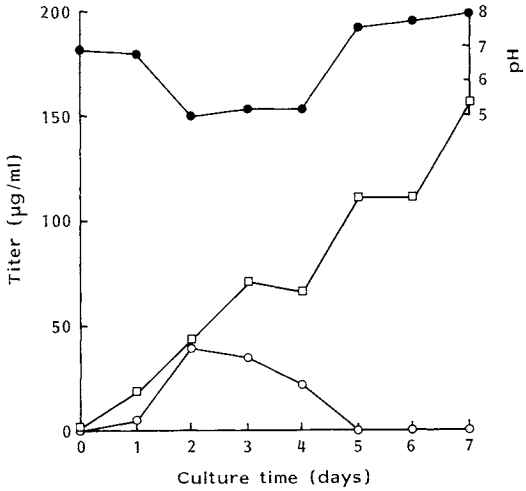
Shift (ppm)	Multiplicity	Assignment
190.0	s	4
147.8	s	2
135.8	d	5
112.4	d	6
88.3	d	4′
84.8	d	1′
73.7	d	2′
69.4	d	3′
60.3	t	5′

Table 2. Antimicrobial spectrum of 4-thiouridine.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	25
S. epidermidis	>100
Bacillus subtilis PCI 219	25
B. megaterium ATCC 14945	>100
Micrococcus luteus ATCC 9341	>100
Escherichia coli NIHJ	>100
Klebsiella pneumoniae PCI 602	100
Proteus morganii IFO 3168	>100
Pseudomonas aeruginosa IFO 3445	>100
Salmonella paratyphi	>100
Enterobacter aerogenes ATCC 13048	>100
Serratia marcescens GN 6485	>100
Shigella sonnei	>100
Mycobacterium smegmatis ATCC 607	>100
Candida albicans NIH 3147	>100

Fig. 2. Time course of production of 4-thiouridine and 4-thiouracil by *Streptomyces libani* NK-110134.

○ 4-Thiouridine, □ 4-thiouracil, ● pH.



filtered beer.

The product was analyzed using FD-MS, which showed M^+ at *m/z* 260. The carbon NMR spectrum (DMSO-d_6, internal TMS) was obtained on a Jeol (JNM-GX400) spectrometer. The data are listed in Table 1. The isolated product was not separable from authentic 4-thiouridine (Aldrich) on Merck silica gel TLC with $CHCl_3$-MeOH (6:1). The ultraviolet, infrared and ^1H NMR spectra were consistent with those expected for 4-thiouridine.

Antibiotic activity was evaluated using agar diffusion tests. 4-Thiouridine was found to have weak activity against some Gram-positive bacteria (Table 2). The antibiotic was tested for *in vitro* cytotoxicity against HeLa S_3 cells showing an IC_{50} value of 2.5 μg/ml.

The taxonomic identification of the organism was carried out and strain NK-110134 was compared to *S. libani* subsp. *soldani* (NRRL 8174) which produced 4-thiouracil[4]. These strains were completely identical in respect to color, cultural characteristics, morphology, physiological properties and utilization of carbohydrates. A time course of the production of 4-thiouridine is shown in Fig. 2. The titer was estimated by HPLC-analysis. Strain NK-110134 also produced 4-thiouracil which had no antimicrobial activity against *B. subtilis*.

Acknowledgments

We wish to thank Mr. M. SATO for NMR measurements and Mr. S. INADA for FD-MS.

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