# MICROBIAL CONVERSION OF ANTHRACYCLINE ANTIBIOTICS

## I. MICROBIAL CONVERSION OF ACLACINOMYCIN B TO ACLACINOMYCIN A

TATSUO HOSHINO, YUZURU SEKINE and AKIKO FUJIWARA

Department of Microbiology and Chemotherapy, Nippon Roche Research Center, 200 Kajiwara, Kamakura City, Kanagawa 247, Japan

(Received for publication June 28, 1983)

*Streptomyces galilaeus* OBB-111 and its blocked mutant were found to convert aclacinomycin B and related anthracycline glycosides of the B type to the corresponding A-type glycosides. Only the cell fraction of cultures of *S. galilaeus* OBB-111 was capable of catalyzing the reaction. The activity was associated with growth but dissappeared before the start of rapid production of aclacinomycins A and B.

According to OKI *et al.*[1], aclacinomycin B is formed by *Streptomyces galilaeus* MA-144-M1 from aclacinomycin A through aclacinomycin Y. The process involves an enzymatic oxidation and non-enzymatic addition of a hydroxyl group to the enone system generated at the terminal sugar. An oxidoreductase involved in the conversion of aclacinomycin A to Y was purified and characterized from the culture filtrate by YOSHIMOTO *et al.*[2] This enzyme did not cause any change in aclacinomycin B.

We have found that aclacinomycin B is converted to aclacinomycin A microbiologically. Activity was found in the cultured broth of *S. galilaeus* OBB-111, a producer of aclacinomycins, auramycins and sulfurmycins[3], and of a blocked mutant, OBB-111-848. These strains also catalyzed the conversion of other similar glycosides including auramycin B, sulfurmycin B, 1-hydroxyauramycin B[4], 1-hydroxysulfurmycin B[4] and cinerubin B to the corresponding A-type glycosides. The structures of these anthracycline glycosides are shown in Table 1.

This paper describes the initial characterization of the conversion activity of *S. galilaeus* OBB-111-848 and OBB-111.

### Materials and Methods

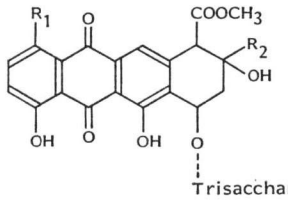
Anthracyclines

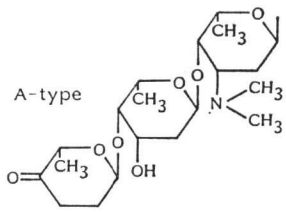
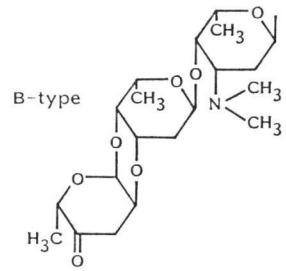
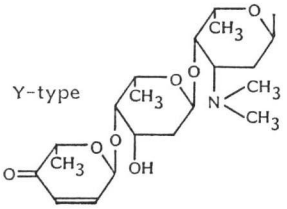
Auramycins A and B and sulfurmycins A and B were obtained from cultures of *S. galilaeus* OBB-111-610[3] and aclacinomycins A and B from OBB-111. 1-Hydroxyauramycins A and B, 1-hydroxysulfurmycins A and B, and cinerubins A and B were obtained from cultures of *S. melanogenes* AC-180[4].

Microbial Conversion

A loopful of spores from an agar slant of *S. galilaeus* OBB-111-848 (or OBB-111) was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of a medium consisting of 2% glucose, 2% soluble starch, 1% Pharmamedia (Traders Oil Mill, U.S.A.), 0.1% $K_2HPO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% NaCl and 0.3% $CaCO_3$. The flask was incubated at 27°C for 3 days on a rotary shaker (180 rpm). A 5-ml portion of the culture thus obtained was added to 100 ml of fresh medium and incubated for 1~4 days. The culture was centrifuged at 10,000×*g* for 10 minutes to separate the cells. The cells were washed with physiological saline, resuspended in 100 ml of the saline and added to a 500-ml Erlenmeyer flask. The culture filtrate was also tested for conversion activity. To the flask was added the substrate anthracycline dissolved in 0.2 ml of dimethyl sulfoxide and the mixture was incubated at 27°C on a rotary

Table 1. Structures of anthracycline glycosides.




| | Aglycone | | Trisaccharide |
|---|---|---|---|
| | $R_1$ | $R_2$ | |
| Auramycin | H | $CH_3$ |  |
| Sulfurmycin | H | $CH_2COCH_3$ | |
| Aclacinomycin | H | $CH_2CH_3$ |  |
| 1-Hydroxyauramycin | OH | $CH_3$ | |
| 1-Hydroxysulfurmycin | OH | $CH_2COCH_3$ | |
| Cinerubin | OH | $CH_2CH_3$ |  |





shaker (180 rpm). A sample was withdrawn at appropriate time intervals and analyzed.

Time Course of B to A Conversion

To cell suspensions prepared from 100 ml of 2-day cultures of *S. galilaeus* OBB-111-848 as described above, 5 mg of auramycin B, sulfurmycin B or aclacinomycin B was added. A sample was withdrawn after 1, 2, 3 and 4 hours of incubation and analyzed.

Analyses

Thin-layer chromatography was carried out on silica gel (Kieselgel 60, $F_{254}$; E. Merck & Co.). The solvent system was benzene - ethyl acetate - methanol - formic acid - $H_2O$ (5: 5: 1.5: 1: 0.3). The quantity of each component was determined by a Chromatoscanner CS-910 (Shimadzu) at 430 nm.

The glucose concentration in the broth was determined with a Glucose Analyzer (Beckman model 2).

Cell growth was estimated from the UV absorbance at 260 nm of the 5% trichloroacetic acid-soluble fraction of cells from 1 ml of the culture.