NOTES

MICROBIAL TRANSFORMATION OF RUBEOMYCIN A TO RUBEOMYCIN B

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Carbonyl reduction by microorganisms and mammalian cells is a well known phenomenon^{1~5)}. This reduction is frequently an enantio selective reaction⁶⁾. This phenomenon is advantageous for the synthesis or conversion of antibiotics and other biologically active materials. In this report, we deal with carbonyl reduction in rubeomycin A (corresponded to carminomycin $II^{(8)}$) using yeasts.

Thirty strains of yeast were grown in test tubes $(18 \times 200 \text{ mm})$ containing 5 ml of medium comprised of 2% glucose, 1% peptone (Polypepton, Daigo Eiyo), 0.01% yeast extract and deionized water (pH 7.0) at 28°C for 20 hours with continuous shaking. Then, 0.5 ml of rubeomycin A solution (1 mg/ml) was added to each test tube and incubated for a further 20 hours under the same conditions. After the incubation, each broth was adjusted to pH 8.5 with sodium bicarbonate and the antibiotic complex extracted with

Table	1.	Res	ults	of	microbiological	reduction	of
rube	eom	ycin	A t	o ru	ibeomycin B.		

Microorganisms	Rubeomycin content (µg/ml)		Reduction
	A	В	
Torulopsis candida	90	8	8.2
Rhodotorula glutinis IFO 0389	24	84	77.8
Rhodosporidium toruloides IFO 0559	85	14	14.1

chloroform. The extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residues were chromatographed on silica gel plate (Merck, Kieselgel 60 F_{254}) in a solvent system containing chloroform - methanol - acetic acid (80: 20: 4) and analyzed with a TLC scanner (Shimadzu CS-920) at 493 nm.

The yeasts in Table 1 were found to reduce rubeomycin A to rubeomycin B (corresponded to 4-hydroxybaumycinol A_1 or $A_2^{(0)}$).

The most effective yeast, *Rhodotorula glutinis* IFO 0389 was transferred to a 500-ml shaking flask containing 100 ml of medium and the flask was incubated under the conditions noted above. After this pre-incubation, the cultured broth was centrifuged and the yeast cells were resuspended in the same volume of fresh medium containing 2% glucose, 1% peptone and 1/15 M phosphate buffer. Then, 1 ml of rubeomycin A in acetic acid solution was added to the 9 ml of yeast cell suspension in a L-form tube and incubated at 35°C with shaking for various times. After the



Fig. 1. Effects of temperature on reduction. Initial concentration of rubeomycin A is

100 μ g/ml. The pH value of reaction mixture is 7.0.



Fig. 2. Effects of pH on reduction. Initial concentration of rubeomycin A is 100 μ g/ml. Incubation temperature is 35°C.



incubation, the reaction mixture was poured into an equal volume of acetone to stop the enzyme reaction. The centrifuged supernatant of the above mentioned reaction mixture was analyzed by HPLC (Water's HPLC System; column: Radialpak B; solvent: chloroform - methanol acetic acid - water - triethylamine (70: 16: 10: 4: 0.01); detector: Shimadzu Fluorescence SpecFig. 3. Recovery of rubeomycin B from the reaction mixture of different initial concentrations of rubeomycin A.

The pH value of reaction mixture is 7.0. Incubation temperature is 35° C.



tromonitor RF-530, emission: 538 nm, excitation: 468 nm) to determine the quantities of rubeomycin A and rubeomycin B.

The experimental results are summarized in Figs. 1, 2 and 3. The optimum pH and temperature for this reduction was pH $7.0 \sim 8.0$ and $30 \sim 40^{\circ}$ C, respectively.

As shown in Fig. 3, in the cases of dosages of less than 30 mg in 10 ml of reaction mixture, more than 70% of the added rubeomycin A was transformed to the rubeomycin B reduction product after 4 hours incubation.

The NMR spectrum and other chemical properties of the obtained reduction compound were in fair agreement with those of authentic rubeomycin B that had been previously reported⁷).

Rubeomycin A_1 was also converted to rubeomycin B_1 .

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