Results and Discussion

Conversion of Various Anthracyclines

When auramycin A, sulfurmycin A, aclacinomycin A, 1-hydroxyauramycin A, 1-hydroxysulfurmycin A and cinerubin A were subjected to microbial conversion by the filtrate fraction of a *S. galilaeus* OBB-111-848 culture, they were converted to the corresponding glycosides of types Y and B, as shown in Table 2. The cell fraction showed no conversion activity for these antibiotics. The results were consistent with those reported by YOSHIMOTO *et al.*[2] with *S. galilaeus* MA-144-N1. It should be noted, however, that blocked mutants of their strain were reported to be devoid of such activity, while the blocked mutant of our strain retained the activity.

When auramycin B, sulfurmycin B, aclacinomycin B, 1-hydroxyauramycin B, 1-hydroxysulfurmycin B and cinerubin B were subjected to the same test as described above, they were not changed by the filtrate fraction of *S. galilaeus* OBB-111-848 but were converted to the corresponding A-type glycosides by the cell fraction. No formation of corresponding glycosides of the Y type was seen. This is the first observation of microbial conversion of B-type glycosides to those of the A-type.

Time Course of B to A Conversion

The change in ratio of glycoside A to B during incubation is shown in Table 3. About 90% of the B-type glycosides were converted to those of the A-type within 2~3 hours incubation. Structural difference among the three aglycones did not influence the conversion rate or the velocity of the reaction.

Table 2. Microbial conversion of various anthracyclines.

Substrate*		Cells		Culture filtrate	
	Rf**	Product	Rf	Product	Rf
AM-A	0.25	No change		AM-Y	0.28
				AM-B	0.32
HAM-A	0.25	〃		HAM-Y	0.28
				HAM-B	0.32
SM-A	0.20	〃		SM-Y	0.23
				SM-B	0.30
HSM-A	0.20	〃		HSM-Y	0.23
				HSM-B	0.30
ACM-A	0.32	〃		ACM-Y	0.39
				ACM-B	0.51
C-A	0.32	〃		C-Y	0.39
				C-B	0.51
AM-B	0.32	AM-A	0.25	No change	
HAM-B	0.32	HAM-A	0.25	〃	
SM-B	0.30	SM-A	0.20	〃	
HSM-B	0.30	HSM-A	0.20	〃	
ACM-B	0.51	ACM-A	0.32	〃	
C-B	0.51	C-A	0.32	〃	

* Abbreviations: AM=auramycin, HAM=1-hydroxyauramycin, SM=sulfurmycin, HSM=1-hydroxysulfurmycin, ACM=aclacinomycin, C=cinerubin.

** See Materials and Methods for thin-layer chromatographic system.

Change in Conversion Activity during Cultivation

The change in ability of *S. galilaeus* OBB-111-848 cells to convert B-type to A-type glycosides was examined at different growth stages. The results are shown in Fig. 1. The activity appeared in association with the growth of the

Fig. 1. The change in the conversion activity (B→A) of the cells during cultivation of *S. galilaeus* OBB-111-848.

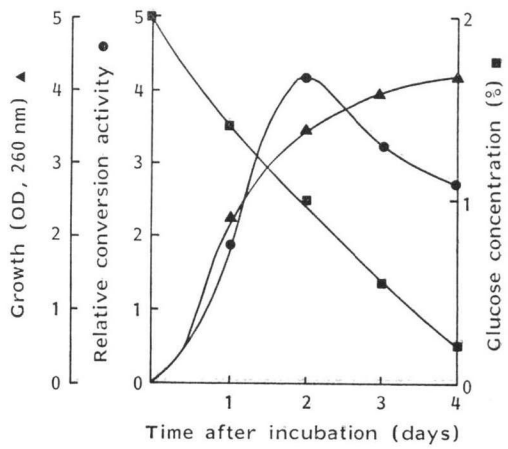

Fig. 2. Relationship between the production of aclacinomycins A and B and the conversion activity (B→A) of *S. galilaeus* OBB-111.

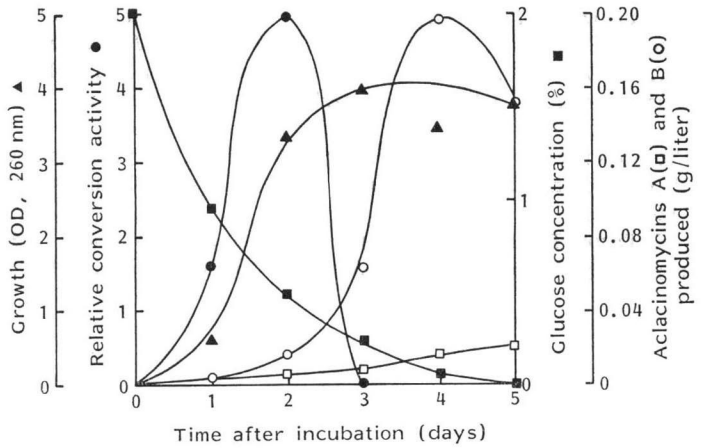

Table 3. Time course for conversion of glycoside B to glycoside A by *S. galilaeus* OBB-111-848.

Substrate	Components in product	Ratio (%)				
		0	1 hour	2 hours	3 hours	4 hours
Auramycin B	Auramycin B	100	45.1	20.7	8.9	4.9
	Auramycin A	0	54.9	79.3	91.1	95.1
Sulfurmycin B	Sulfurmycin B	100	30.9	10.2	8.1	7.8
	Sulfurmycin A	0	69.1	89.8	91.9	92.2
Aclacinomycin B	Aclacinomycin B	100	40.5	16.5	8.1	6.0
	Aclacinomycin A	0	59.5	83.5	91.9	94.0

strain, reaching a maximum in the early stationary phase and decreasing gradually thereafter.

Relationship between Production of Aclacinomycins A and B and the Conversion Activity of *S. galilaeus* OBB-111

The ability to convert B-type to A-type glycosides was also found in the parent strain, *S. galilaeus* OBB-111. We examined the relationship between antibiotic production and conversion activity in this strain. As shown in Fig. 2, the conversion activity was associated with growth, as in the case of strain OBB-111-848, but it disappeared before the start of rapid production of aclacinomycins A and B. On the other hand, oxidoreductase activity which catalyzed the conversion of aclacinomycin A to Y was found to increase in parallel with production of the antibiotics (data not shown), as reported by YOSHIMOTO *et al.*[2] These results explain the difference in production levels of aclacinomycins A and B shown in Fig. 2 because only the activity converting aclacinomycin A to B through aclacinomycin Y was present during the antibiotic production phase. However, almost all of the aclacinomycins were recovered from the cells whereas the oxidoreductase was found only in the culture filtrate. It remains to be determined where aclacinomycin A is converted to aclacinomycin B.

So far, we have not succeeded in preparing an *in vitro* system for the microbial conversion of B-type glycosides to those of the A-type.

References

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