BIOCONVERSIONS OF SAFRAMYCIN A SPECIFIC TO SOME GENERA OF ACTINOMYCETES

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Saframycin A is an antibiotic with twin *N*-heterocyclic quinone moieties in its basic skeleton. Its biological activity and mechanism of action have been reported[1~5].

During studies on microbial conversions of saframycin A, reduction of the ketone group of the pyruvoylamine side chain was observed and further studies revealed that the reduction occurred specifically in some genera of actinomycetes. In this note, we relate the bioconversion pattern of saframycin A by actinomycetes with their taxonomy.

Actinomycetes maintained in our laboratory were grown at 27°C for 3 to 4 days in a medium (pH 7.2) consisting of 1.0% glycerol, 0.5% peptone, 0.3% yeast extract, 0.3% meat extract and 0.5% K_2HPO_4.

The mycelium from cultures in late exponential growth phase was centrifuged, washed and sonicated for use as a microbial conversion agent. Conversion, detection and extraction of the products were carried out by the procedures described by TAKAHASHI *et al.*[6]

In the course of this work, we found that a soil isolate, identified as *Rhodococcus* sp., can bring about conversion of saframycin A to three products, saframycins AR_1, AR_2 and AR_3. The structures of the products were determined to be 25-dihydrosaframycin A, saframycin B (21-decyanosaframycin A) and 25-dihydro-21-decyanosaframycin A, respectively[6]. Other genera of soil isolates showed different conversion patterns. This prompted us to study the relationship between the conversion pattern by various actinomycete genera and classification of the order *Actinomycetales*.

Four hundred and eighty four actinomycetes including 8 *Rhodococcus* species, 4 *Mycobacterium* species, 5 *Nocardia* species, 417 ISP (International Streptomyces Project) *Strepto-*

Table 1. Microbial conversion of saframycin A by various actinomycetes.

Cell wall type[a]	Genera	No. of tested species[b]	Bioconversion type[c]
I	*Streptomyces*	417	S-type (98)[d]
			N-type (2)
	Streptoverticillium	15	S-type (100)
	Microellobosporia	4	S-type (100)
II	*Micromonospora*	15	S-type (93)
			N-type (7)
	Actinoplanes	7	N-type (57)
			S-type (43)
	Ampullariella	4	M-type (75)
			S-type (25)
III	*Streptosporangium*	3	N-type (67)
			S-type (33)
	Actinobifida	2	N-type (100)
IV	*Nocardia*	5	N-type (100)
	Rhodococcus	8	N-type (100)
	Mycobacterium	4	M-type (100)

a) Cell wall type by LECHEVALIER and LECHEVALIER (1970)[8].
b) Species from ISP *Streptomyces*.
c) S-type: *Streptomyces* type, N-type: *Nocardia* type, M-type: *Mycobacterium* type.
d) Percent of species.

Fig. 1. Typical saframycin A bioconversion patterns by three representative actinomycete genera (after extraction with ethyl acetate, the conversion products were developed using silica gel TLC[8]).

A: saframycin A, AR_1: saframycin AR_1, AR_2: saframycin AR_2, AR_3: saframycin AR_3.

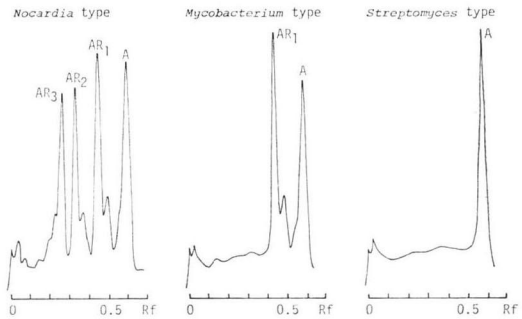
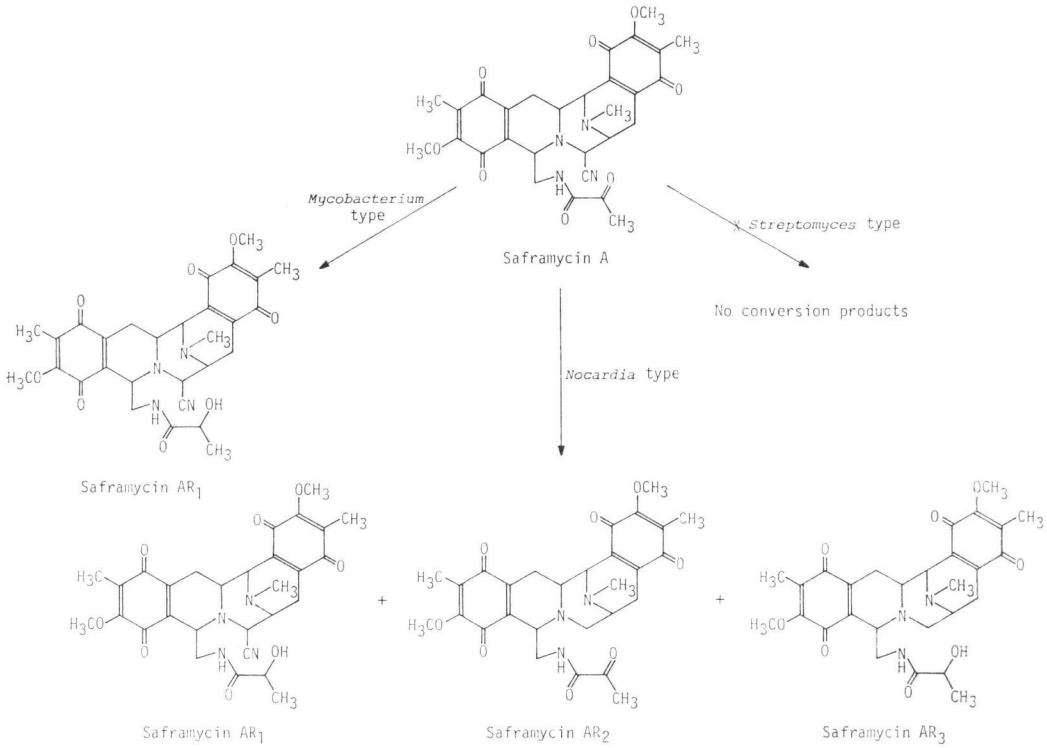


Fig. 2. Saframycin A bioconversion products produced by actinomycetes.



myces[7], 15 *Streptoverticillium* species, 2 *Actinobifida* species, 4 *Ampullariela* species, 3 *Streptosporangium* species, 7 *Actinoplanes* species, 4 *Microellobosporia* and 15 *Micromonospora* species were tested for their ability to convert saframycin A. The results are shown in Table 1. They indicate that the genera of order *Actinomycetales* can be divided into the following three groups on the basis of their bioconversion patterns: (I) *Nocardia* type, (II) *Mycobacterium* type and (III) *Streptomyces* type (Fig. 1). The reactions are depicted schematically in Fig. 2.

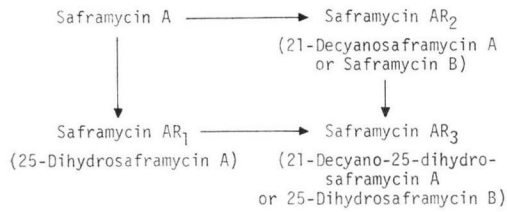
The *Nocardia* type includes *Rhodococcus*, *Nocardia*, some species of *Actinoplanes* and *Streptosporangium*. These produced three bioconversion products, saframycins AR_1, AR_2, and AR_3.

The *Mycobacterium* type includes *Mycobacterium* and some species of *Ampullariella*. These produced only saframycin AR_1. The *Streptomyces* type includes *Streptomyces*, *Streptoverticillium*, *Microellobosporia*, one species of *Micromonospora*, and all of the genera belong to the cell wall type I of LECHEVALIER and LECHEVALIER[8]. No bioconversion products were observed in this group of actinomycetes.

We tested 417 ISP *Streptomyces* species, and 9 of them showed the same conversion pattern as the genus *Nocardia* (*Nocardia* type). Some of these exceptional *Streptomyces*, such as *S. aerocolonigenes* ISP 5034, *S. orientalis* ISP 5040, *S. autotrophicus* ISP 5011, *S. salmonicida* ISP 5472, and *S. mediterranei* ISP 5501, are reported to have been misidentified by the original authors[9]. Our preliminary studies by cell wall analysis of the other exceptional *Streptomyces* species showed that 5 had meso-type DAP[10]. Further detailed taxonomic study of these species is necessary.

Although more than 90% of *Micromonospora* species showed the *Streptomyces* type bioconversion pattern, only one out of 15, *i.e.*, *M. purpureae*, showed a *Mycobacterium* type bioconversion pattern. Detailed studies of this exceptional species will be attempted. An examination of actinomycete genera such as *Actinoplanes*, *Ampullariella*, *Streptosporangium* and *Actinobifida* is also underway to establish the relationship between their taxonomic position and their saframycin A bioconversion pattern.

Fig. 3. Saframycin A bioconversion routes by actinomycetes.



In considering an over all results of saframycin A bioconversion, two routes seem possible (Fig. 3). The first is a reduction of the C-25 ketone of the pyruvoylamine side chain to a carbinol (giving saframycin AR_1) followed by a reductive eliminaton of the C-21 cyanide (giving saframycin AR_3). This route is supported by the observation that, when saframycin AR_1 was used as a substrate instead of saframycin A, the only conversion product was saframycin AR_3.

The second possible route is a reductive elimination of C-21 cyanide (giving saframycin AR_2) followed by reduction of the C-25 ketone (giving saframycin AR_3).

Based on these possibilities, *Mycobacterium* has only one enzymatic activity which is capable of catalyzing the reduction of saframycin A to saframycin AR_1, or of saframycin AR_2 to saframycin AR_3. On the other hand, *Nocardia* and *Rhodococcus* have one additional enzymatic activity which is capable of catalyzing a reductive elimination of cyanide, leading to the production of saframycin AR_2 from saframycin A, or of saframycin AR_3 from saframycin AR_1. Although, in the present experiments, sonicated cell suspensions were used to catalyze the conversions, the same activity was found in crude cell-free enzyme preparations.

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