Ninety-five streptomycin-nonproducing mutants derived from *Streptomyces griseus* FT-1 by UV-irradiation could be classified into major two classes by cosynthesis tests. Class I mutants (42 strains) were mutants blocked in the pathway of streptomycin biosynthesis while class II mutants (49 strains) required a factor for streptomycin biosynthesis which was excreted by the parental or class I mutant strains. The factor could be replaced by synthetic A-factor (2S-isocapryloyl-3S-hydroxymethyl-7-butyrolactone) which restored both streptomycin biosynthesis and spore formation in the class II mutants. A-Factor deficient mutants were obtained from several strains of *S. griseus* and *S. bikiniensis* at high frequency by treatment with acridine orange or incubation at high temperature. A mutant whose streptomycin biosynthesis was independent of A-factor deficiency was found. The production of A-factor was distributed among various species of actinomycetes.

A-Factor (autoregulating factor) was detected by KHOKHLOV et al.1-5) from *Streptomyces griseus* as a factor stimulating streptomycin production by this organism and its structure, 2S-isocapryloyl-3S-hydroxymethyl-7-butyrolactone, was determined by chemical synthesis.6) A-Factor deficient mutants of *Streptomyces griseus* lose not only streptomycin productivity but also their ability to form spores. Addition of a small quantity of the factor to the mutant restores both of these abilities. Other substances inducing antibiotic production or morphological differentiation have been isolated from streptomycetes. YANAGIMOTO et al.7) reported that a factor isolated from *S. virginiae* stimulated production of staphylocycin by this organism. POGELL et al.8,9) reported that pamamycin, an antibiotic produced by *S. alboniger* stimulated its aerial mycelia formation. SCRIBNER et al.10) and BIRÓ et al.11) also reported presence of substance which influenced spore formation.

Genetic studies with streptomycetes have indicated numerous pleiotropic mutations as well as instability of various phenotypic characters such as antibiotic production, formation of aerial mycelia and nutrient auxotrophy.8,12-20) Treatment of streptomycetes with acridine dyes or at high temperature frequently causes loss of various phenotypic characters with coincidental disappearance of plasmids. In *S. coelicolor* A3(2), the direct involvement of plasmids in the production of methylenomycin A (SCP1 plasmid)21,22) and streptocin A, B and amrumycin (SCP2 plasmid)23) has been confirmed. Recently, the involvement of transposable genetic determinants was suggested by the finding of pleiotropic mutations of chloramphenicol resistance linked with arginine auxotrophy in *S. coelicolor* A3(2),24) formation of aerial mycelia linked with various phenotypes including arginine auxotrophy in *S. alboniger*25) and streptomycin resistance linked with aerial mycelia formation and streptomycin production in *S. griseus* and *S. bikiniensis*.26) It would appear that most of the unstable genes in actinomycetes have pleiotropic features.

In the course of genetic studies on streptomycin production by *S. griseus*, we found that strepto-
mycin-nonproducing mutants derived by UV-irradiation could be classified into two distinct cosynthesis groups; mutants of one group restored streptomycin productivity in the presence of a factor which was excreted by mutants of the other group. Further investigations revealed that the former group were A-factor deficient. These mutants lost both spore forming ability and streptomycin productivity due to the multifunctional effect of A-factor. Studies suggested that an extrachromosomal or transposable genetic element was involved in A-factor synthesis in *S. griseus*. The mechanism in which A-factor and its genetic determinant are involved seems to be one of typical mechanism in the pleiotropic mutations in streptomycetes. In this paper, we describe the isolation and characterization of a group of streptomycin-nonproducing mutations of *S. griseus*.

**Materials and Methods**

**Microorganisms**

*Streptomyces griseus* FT-1, which produces streptomycin, was obtained from Meiji Seika Kaisha, Ltd., Tokyo. *S. griseus* 2247 was provided by R. Nomi, Hiroshima University. *S. coelicolor* A3(2) was provided by D. A. Hopwood, John Innes Institute. Other strains of actinomycetes used in this work were stock cultures in our laboratory or were obtained from type culture collections of Institute for Fermentation, Osaka, Kaken Chemical Co., Tokyo, and Meiji Seika Kaisha, Ltd. *Bacillus subtilis* ATCC 6633 was used for bioassay of streptomycin.

**Media.**

Nutrient agar (Difco Laboratories) was used for tests of the production of streptomycin. The liquid medium for curing experiments was glucose - meat extract - peptone medium containing 1% glucose, 0.2% yeast extract, 0.2% meat extract, 0.4% peptone, 0.5% NaCl and 0.025% MgSO₄·7H₂O, pH 7.0, and for production of streptomycin (Chuken medium) 2% glucose, 0.1% meat extract, 1% soy bean flour, 0.4% KCl, 0.25% dry yeast, 0.5% (NH₄)₂SO₄, 0.4% CaCO₃ and 0.02% K₂HPO₄, pH 7.0. V-8 agar medium was used to examine spore forming ability.

**Materials**

A-Factor was provided by A. S. Khokhlov, M. M. Shemyakin Institute of Bioorganic Chemistry, and by K. Mori, this department. Streptidine, O-α-L-dihydrostreptose (1→4) streptidine and mannosidostreptomycin were obtained from Meiji Seika Kaisha, Ltd.

**Isolation of Streptomycin-nonproducing Mutants**

For the isolation of mutants deficient in streptomycin synthesis, the procedure described by Ichikawa et al. was modified as follows. A spore suspension of *S. griseus* FT-1 in distilled water (10⁶/ml) was irradiated with ultraviolet (UV) light to give 0.1~1% of surviving cells and the irradiated spores were plated on nutrient agar and incubated at 28°C for 3~4 days. The colonies were transferred with a toothpick onto agar pieces (5 mm diameter) which were separately placed in a Petri dish. Petri dishes were incubated at 28°C for 3 days. After the incubation, the agar pieces were placed on bioassay plates and incubated at 37°C for 12~18 hours. The colonies which had no inhibitory zone against *B. subtilis* ATCC 6633, were isolated as streptomycin-nonproducing mutants.

**Cosynthesis**

Two streptomycin-nonproducing mutants to be tested were transferred from a master plate onto a nutrient agar plate with a toothpick, separated by 1 cm, and incubated at 28°C for 2~3 days. Nutrient agar containing *B. subtilis* ATCC 6633 was overlaid. After incubation at 37°C for 12~18 hours, the cosynthesis of streptomycin could be detected.

**Plate Assay of A-Factor**

The A-factor deficient mutant of *S. griseus* FT-1 was cultured in nutrient broth (Difco Laboratories) at 28°C with shaking. After 2 days, the culture broth was homogenized with a glass homogenizer to obtain a uniform cell suspension. An aliquot of the cell suspension was mixed with a 100-fold volume
of nutrient soft agar solution (agar, 0.5%) at a temperature as low as possible and the mixture overlaid on the nutrient agar base plate (agar, 2%). A paper disc containing A-factor was placed onto the agar plate. After the incubation at 28°C for 2 days, nutrient soft agar (agar, 1%) containing *B. subtilis* ATCC 6633 was overlaid. The plate was further incubated at 37°C for 18~24 hours and the diameter of the inhibitory zone was measured. It was possible to measure the concentration of A-factor by comparing with a standard curve obtained from a control performed simultaneously. A-Factor in culture broth was extracted with an equal volume of chloroform prior to the assay.

**Curing Experiments**

Samples (0.1 ml) of two day cultures of the parental *Streptomyces* strains were inoculated into 10 ml of glucose - meat extract - peptone medium in test tubes (21 mm diameter) in the presence of curing agents, *i.e.*, acriflavin (15 μg/ml), acridine orange (20~30 μg/ml) or ethidium bromide (15 μg/ml) and cultured aerobically by shaking at 30°C. After 3~4 days cultivation, the culture broth was homogenized with a glass homogenizer and plated onto nutrient agar plates. A culture without curing agents was made at 35~37°C. Streptomycin-nonproducing mutants were isolated as described above.

**Thin-layer Chromatograph and Bioautography**

A-Factor could be extracted with chloroform from culture filtrates. Crude extracts were applied to thin-layer silica gel plates (Merck), the solvent was chloroform - methanol (50:1, v/v). After development, the dried silica gel plates were placed on A-factor assay plates described above and kept at 4°C for 1 hour prior to the incubation. The silica gel plates were removed and A-factor detected by the same procedure as used for the plate assay.

**High Performance Liquid Chromatography**

The high performance liquid chromatograph system consisted of a pump (Twinkle-Jasco, Japan Spectroscopic Co., Tokyo) and an autoanalyzer system (Technicon Instruments Corp., New York). Analysis was performed by using a column (30 cm × 3 mm i.d.) packed with CK10S (Mitsubishi Chemical Industries, Ltd., Tokyo). The mobile phase contained 1.1 m NaCl and 0.1 m Na₂HPO₄, pH 8.2. The operating conditions were as follows: flow rate, 0.7 ml/minute; pressure, about 98 kg/cm² and column temperature, 60°C. The α-naphthol reaction was used for detection of the guanidine group in streptomycin and the pinkish color detected at 530 nm.

**Results**

Isolation and Classification of Streptomycin-nonproducing Mutants

*Streptomyces griseus* FT-1 produces streptomycin. After UV-irradiation of spore suspensions, 95 streptomycin-nonproducing mutants were obtained out of 6476 colonies tested (ca. 1.5%). Cosynthesis of streptomycin by pairs of these 95 mutants was examined as described above. Some pairs cosynthesized streptomycin when cultured in proximity on the nutrient agar surface. In Fig. 1, an example is shown. The streptomycin-nonproducing mutant No. 2 produced streptomycin when grown with the streptomycin-nonproducing mutant No. 55 or the parent strain, but No. 55 could not produce streptomycin when grown with No. 2 or the parent strain. Thus, No. 55 and the parent strain apparently excrete a factor or intermediate
which was essential for No. 2 to synthesize streptomycin. When all 95 streptomycin-nonproducing mutants were examined by this method, it was found that most fell into two classes. The class I mutants, represented by No. 55, failed to restore streptomycin production in pair cultures with members of class II or the parent strain, but could restore antibiotic production of the class II mutants. The class II mutants, represented by No. 2, restored streptomycin producing ability when cultured with the class I mutants or the parent strain, but not restore production to members of class I. The cross-feeding relationship between these two classes were non-reciprocal. Among the 95 streptomycin-nonproducing mutants, 42 belonged to class I and 49 to class II. Four strains such as No. 50, failed to regain streptomycin producing ability in co-culture with all other mutants or the parent strain, nor could they restore production to the other mutants. Therefore, these mutants (class III) appear to be double mutants of class I and class II. The mutants of class II and III did not form spores on V-8 agar medium while the class I mutants varied.

Chromatographic Analysis of Culture Filtrates of Streptomycin-nonproducing Mutants

In order to characterize substances involved in cosynthesis, culture filtrates of streptomycin-nonproducing mutants of the three classes were analyzed by high performance liquid chromatography (HPLC). Typical HPLC patterns are shown in Fig. 2. The mutants No. 55, 56 and 65 belonged to class I. No. 55 accumulated streptidine, and No. 56 accumulated O-α-L-dihydrostreptose(1→4)streptidine. Both streptidine and O-α-L-dihydrostreptose(1→4)streptidine are intermediates in streptomycin biosynthesis. Although No. 65 did not accumulate any intermediates, it produced streptomycin in the presence of streptidine or O-α-L-dihydrostreptose (1→4) streptidine; similar to a mutant isolated by NAGAOKA and DEMAIN [27] which requires streptidine for production of streptomycin. From these results, it is clear that class I mutants are mutants blocked in the pathway of streptomycin biosynthesis.

Fig. 2. High performance liquid chromatographic patterns of culture filtrates of streptomycin-nonproducing mutants.

1, mannosidostreptomycin. 2, streptomycin. 3, O-α-L-dihydrostreptose (1→4) streptidine. 4, streptidine.

Fig. 3. Plate assay and quantitation of stimulation of streptomycin production in A-factor deficient mutant of S. griseus FT-1 by A-factor.

(A) Demonstration of plate assay. (B) Standard curve. S-Form isomer (○) and the mixture containing S-and R-form isomers in the ratio of 1:2 (●).
On the contrary, the class II mutants neither accumulated intermediates of streptomycin biosynthesis, nor produced streptomycin in the presence of streptidine or \(O-\alpha-L\)-dihydrostreptose \((1\rightarrow4)\) streptidine. Therefore, the class II mutants were different from those of the class I. The HPLC pattern of the class III mutant No. 50 was similar to those of class II mutants. The fact that all the class II and III mutants simultaneously lost spore forming ability in addition to antibiotic production suggested that any substance involved in the cosynthesis was not an intermediate in streptomycin biosynthesis. One possibility is that these mutants would respond to A-factor as described by Khokhlov et al.\(^{1-5}\)

Isolation and Identification of a Factor Stimulating Streptomycin Biosynthesis

The factor stimulating streptomycin biosynthesis in the class II mutants was extracted with chloroform from culture filtrates of \(S.\ griseus\) FT-1 or class I mutants. Biological assays of the stimulating activity were carried out as described in Materials and Methods. Synthetic samples of A-factor, 2S-isocapryloyl-3S-hydroxymethyl-\(\gamma\)-butyrolactone provided by Khokhlov and by Mori, gave identical reactions in the assay at a concentration of 2 ng/disc as shown in Fig. 3. We compared the specific activities using two chemically synthesized substances, which were supplied by Mori.\(^{28}\) It showed that the optically pure S-form\((3S)-(\_\_\_\_\_\_\_)\)isomer of A-factor had 60–80\% higher specific activity in comparison with the mixture containing \(S\) and \(R\) \((3R)-(\_\_\_\_\_\_)\)isomers in the ratio of 1:2. The active material coincided completely with the spot of the synthetic A-factor on thin-layer chromatography with an Rf value of 0.30 in chloroform - methanol \(50:1, v/v\). Thus we conclude that the stimulating factor for the class II mutants is A-factor.

Effect of Curing Agents and High Temperature

The fact that A-factor deficient mutants (class II) were obtained at a frequency of greater than 50\% out of the streptomycin-nonproducing mutants by UV-irradiation suggested the involvement of highly mutable genes in the production of A-factor. To examine the possibility that the gene was extrachromosomal, the cells were treated with putative plasmid curing agents. \(S.\ griseus\) FT-1 was grown in glucose-meat extract - peptone medium in the presence of curing agents or at high temperature. Four hundred colonies from each culture were selected at random and examined for their production of streptomycin and A-factor. As shown in Table 1, acridine orange and high temperature treatment gave many streptomycin-nonproducing colonies, and moreover, most of the streptomycin-nonproducing colonies were A-factor deficient. Other curing agents, acriflavine and ethidium bromide, had weak or almost no effect.

Other strains of \(S.\ griseus\) and \(S.\ bikiniensis\), which produced both streptomycin and A-factor, were also examined. In the case of \(S.\ griseus\) IFO 13189, acridine orange and high temperature treatment

<table>
<thead>
<tr>
<th>Curing agents ((\mu g/ml))</th>
<th>Incubation temperature (^{\circ})C</th>
<th>No. of colonies tested</th>
<th>Total No. of Sm(^{+}) colonies</th>
<th>Class I*</th>
<th>Class II*</th>
<th>Class III*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>30</td>
<td>400</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Acriflavine, 15</td>
<td>30</td>
<td>400</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Acridine orange, 30</td>
<td>30</td>
<td>400</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Ethidium bromide, 15</td>
<td>30</td>
<td>400</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>None</td>
<td>35</td>
<td>400</td>
<td>10</td>
<td>1</td>
<td>7</td>
<td>2</td>
</tr>
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</table>

* Class I, II and III refer to mutants blocked in streptomycin biosynthesis, A-factor deficient mutants and double mutants respectively.
The results of the curing experiments suggest the involvement of an extrachromosomal genetic determinant in the production of A-factor. In order to detect extrachromosomal or plasmid DNA, S. griseus FT-1 was lysed by the method of Horinouchi et al., and subjected to ethidium bromide-cesium chloride dye-buoyant density gradient centrifugation; no satellite bands were detected. In addition plasmid DNA could not be detected by agarose gel electrophoresis in cleared lysates of this strain.

Time Course of Production of A-Factor and Streptomycin by S. griseus

As shown in Fig. 4A, production of A-factor by the parental and class I mutant (No. 55) of S. griseus FT-1 reached a maximum in one day.

Table 2. Effect of acridine orange and incubation at high temperature on streptomycin and A-factor production by S. griseus IFO 13189 and S. griseus 2247.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Acridine orange (µg/ml)</th>
<th>Incubation temperature (°C)</th>
<th>No. of colonies tested</th>
<th>Total No. of Sm− colonies</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. griseus IFO 13189</td>
<td>None</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30</td>
<td>100</td>
<td>11</td>
<td>0</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>37</td>
<td>100</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>S. griseus 2247</td>
<td>None</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>37</td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

Classification of the mutants is the same as in Table 1.
followed by rapid disappearance. Production of streptomycin by the parental strain continued for three days. A similar time course of production of A-factor and streptomycin was obtained with *S. griseus* 2247 (Fig. 4B). As already described above, the A-factor deficient mutant of *S. griseus* 2247 produced streptomycin without supplementing A-factor, however, the amount of streptomycin accumulated was less than that of the parental strain. Although strain 2247 did not have an absolute requirement for A-factor for antibiotic production, a response to the factor remained.

### Table 3. Excretion of stimulators of streptomycin biosynthesis by various actinomycetes.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Stimulators</th>
<th>Strains</th>
<th>Stimulators</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. albus</em> IFO 3195</td>
<td>+</td>
<td><em>S. griseus</em> IFO 12869</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3422</td>
<td>-</td>
<td><em>S. griseoflavus</em> IFO 12372</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3710</td>
<td>-</td>
<td>IFO 13044</td>
<td>+</td>
</tr>
<tr>
<td><em>S. antibioticus</em> IFO 3126</td>
<td>+</td>
<td><em>S. griseoluteus</em> IAM 0060</td>
<td>+</td>
</tr>
<tr>
<td>IFO 12652</td>
<td>-</td>
<td><em>S. griseus</em> IFO 3102</td>
<td>+</td>
</tr>
<tr>
<td>IFO 12838</td>
<td>-</td>
<td>IFO 3237</td>
<td>+</td>
</tr>
<tr>
<td><em>S. aureus</em> IAM 0092</td>
<td>+</td>
<td>IFO 3430</td>
<td>+</td>
</tr>
<tr>
<td><em>S. bikiniensis</em> IFO 13350</td>
<td>+</td>
<td>IFO 12875</td>
<td>+</td>
</tr>
<tr>
<td>IAM 0019</td>
<td>+</td>
<td>IFO 13189</td>
<td>+</td>
</tr>
<tr>
<td><em>S. blastomycticus</em> 295</td>
<td>+</td>
<td>IFO 13304</td>
<td>-</td>
</tr>
<tr>
<td>IFO 12747</td>
<td>-</td>
<td>IAM 0124</td>
<td>-</td>
</tr>
<tr>
<td><em>S. caesecus</em> ISP 5421</td>
<td>+</td>
<td><em>S. hirsutus</em> IFO 12786</td>
<td>+</td>
</tr>
<tr>
<td><em>S. coelicolor</em> A3 (2)</td>
<td>+</td>
<td><em>S. parvus</em> IFO 3388</td>
<td>+</td>
</tr>
<tr>
<td>1023</td>
<td>+</td>
<td><em>S. sindenensis</em> IFO 12915</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3114</td>
<td>-</td>
<td><em>S. viridis</em> IFO 13373</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3176</td>
<td>-</td>
<td><em>S. viridochromogenes</em> IFO 12337</td>
<td>-</td>
</tr>
<tr>
<td><em>S. cyaneofuscatus</em> IFO 13190</td>
<td>+</td>
<td>IFO 12338</td>
<td>+</td>
</tr>
<tr>
<td><em>S. flavolus</em> IAM 0117</td>
<td>+</td>
<td>IFO 12376</td>
<td>-</td>
</tr>
<tr>
<td>IFO 3408</td>
<td>-</td>
<td>IFO 12377</td>
<td>-</td>
</tr>
<tr>
<td><em>S. fradiae</em> ATCC 19609</td>
<td>-</td>
<td><em>S. zaomyceticus</em> IFO 13348</td>
<td>+</td>
</tr>
<tr>
<td>ATCC 21096</td>
<td>+</td>
<td><em>A. citreofluorescens</em> IFO 12853</td>
<td>+</td>
</tr>
<tr>
<td><em>S. globisporus</em> IFO 12208</td>
<td>+</td>
<td><em>A. cyanoalbus</em> IFO 12857</td>
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<tr>
<td>IFO 12209</td>
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<td><em>A. fluorescens</em> IFO 12861</td>
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</tr>
<tr>
<td>IFO 12867</td>
<td>+</td>
<td><em>N. brasiliensis</em></td>
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</table>

Production of A-Factor by Various Strains of Actinomycetes

Various strains of actinomycetes were examined for the production of A-factor or other stimulating factors which would restore streptomycin producing ability to an A-factor deficient mutant of *S. griseus*. Thirty out of 203 strains tested showed distinct A-factor activity (approximately 15%). The activity was detected in 26 of 175 *Streptomyces* spp., 3 of 14 *Actinomyces* spp. and one of 11 *Nocardia* spp. (Table 3). Thin-layer chromatography revealed that these strains produced an active factor having the same Rf value as that of A-factor, indicating A-factor production distributed widely among strains of actinomycetes. Table 3 also shows that A-factor production is characteristic for strains but not for species.

Various strains of actinomycetes producing no antibiotics were examined to test whether they recovered their potential to produce antibiotics by cosynthesis with No. 55, the class I mutant of *S. griseus*; none showed antibiotic production.
Discussion

Our results confirm the role of A-factor in streptomycin synthesis and spore formation in *S. griseus* as previously reported by Khokhlov et al.\(^1\) Our studies show that more than half of the streptomycin-nonproducing mutants of *S. griseus* derived by UV-irradiation were A-factor deficient. Since extremely low concentrations of A-factor (2 ng/disc) are sufficient for the induction of streptomycin synthesis in deficient mutants, cross feeding between the mutagenized colonies may make mutant isolation difficult. The agar piece method used in the present work seems to be efficient in the detection of A-factor deficient mutants.

The optically pure S-form isomer of A-factor gave 60–80% higher activity than that of the 1:2 mixture of the S and R isomers. Assuming that no interference nor stimulation by the R isomer is present, the activity of the S isomer can be calculated to be 2.6 times greater than that of the R isomer. The tendency of A-factor to racemize might be the basis of the positive activity of the latter.

Production of streptomycin by the A-factor deficient mutants could not be detected on the addition of intermediates in streptomycin biosynthesis, i.e., streptidine and *O*-α-L-dihydrostreptose (1→4) streptidine. This suggests that most of the enzymes involved in streptomycin biosynthesis are absent in the mutant cells grown without A-factor. Addition of the factor seems to induce synthesis of these enzymes as well as spore formation. Voronina et al.\(^3\) suggested that A-factor induced the formation of adenosine ciphosphoribose, a breakdown product of nicotinamideadenine dinucleotide phosphate via the phosphatase reaction, which in turn inhibits glucose-6-phosphate dehydrogenase. It was postulated that the decreased level of the enzyme might be the cause of the pleiotropic effect of A-factor. The properties of *S. griseus* 2247 are interesting in this respect; it appears to be a mutant in which streptomycin biosynthesis is independent of A-factor deficiency. Further characterization of the mutation in this strain may give information concerning the mechanism of A-factor action.

Treatment of streptomycin-producing strains of *S. griseus* and *S. bikiniensis* with intercalating dyes or incubation at high temperature led to the appearance of A-factor deficient mutants at high frequency; reversion of these mutations to wild-type was not detected. These facts suggest the involvement of some extrachromosomal or transposable genetic determinant in the production of A-factor. Genetic analysis is now required to determine whether the A-factor gene is linked with chromosomal markers.

The production of A-factor seems to be widely distributed among strains of actinomycetes; Efremenko et al.\(^3\) made similar observations. Although analogue compounds which have A-factor activity might possibly be included in this assay, most of the active substances produced by many strains of actinomycetes had an identical Rf value on thin-layer chromatography with that of A-factor. The addition of A-factor to cultures of various parental strains of actinomycetes had no effect on antibiotic production, but we cannot exclude the possibility that A-factor plays a general regulatory role in actinomycetes. The isolation of mutants requiring A-factor for various phenotypic characters in species of actinomycetes other than *S. griseus* and *S. bikiniensis* would be interesting.

Note Added in Proof

Recently the optically pure R-form isomer of A-factor was synthesized by Dr. K. Mori. We confirmed the result in the text that the activity of S isomer was 2.6 times greater than that of R isomer using the pure S- and R-form in the cosynthesis test described in the text.

Acknowledgements

We thank Dr. A. S. Khokhlov and Dr. K. Mori for providing A-factor. Our thanks are also intended to Dr. D. A. Hopwood for *S. coelicolor* A3 (2), and Dr. R. Nomi for *S. griseus* 2247. We also thank Dr. T. Ishikawa and his coworkers, Meiji Seika Fermentation Technology Laboratories for helpful discussions and valuable technical assistance.

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ing supernatant obtained from mycelium suspension of *Streptomyces griseus. * Agric. Biol. Chem. 30: 296–303, 1966