Microbiology, Vol. 72, No. 6, 2003, pp. 682–685. Translated from Mikrobiologiya, Vol. 72, No. 6, 2003, pp. 770–774.

EXPERIMENTAL ARTICLES

A New Regulatory Function of A-Factor: Stimulation of the Germination of Streptomycete Spores

V. D. Gruzina*, E. V. Gorbatyuk, O. V. Efremenkova*,[1], S. N. Filippova**, G. I. El'-Registan**, and Yu. V. Dudnik***

**Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, ul. Bol'shaya Pirogovskaya 11, Moscow, 119021 Russia*
***Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

Received March 31, 2003

Abstract—Spore germination in streptomycetes was shown to be stimulated by exogenously added A-factor. Agar medium either containing or not containing A-factor was inoculated with spore suspensions of three strains differing in their ability to produce regulators of the A-factor group: *Streptomyces griseus* 773, which produces A-factor and two its lower homologs; *S. coelicolor* A3(2), which forms six Acl-factors (A-factor analogues); and *S. avermitilis* JCM5070, which fails to form regulators of this group. A count of the grown colonies showed that exogenous A-factor stimulated spore germination in strains that were themselves able to synthesize regulators of the A-factor group. In *S. griseus* 773, the number of germinated spores increased by 67% on average after the addition of A-factor to the medium in an amount of 10 μg/ml. In strain *S. coelicolor* A3 (2), the number of germinated spores increased by 75% after the addition of 1 μg/ml of A-factor. During germination of the *S. avermitilis* JCM5070 spores, no changes in the CFU number was observed after the addition of A-factor.

Key words: A-factor, Acl-factors, streptomycetes, spore germination.

A-factor is an endogenous regulator of *Streptomyces griseus* growth that controls morphogenesis and streptomycin biosynthesis [1–4]. Over the 35 years that have passed since the first report devoted to A-factor appeared [3], actinomycetes were shown to also synthesize other substances resembling A-factor in their chemical structure, which are referred to as the "A-factor-group regulators" [4, 5]. These regulators are widespread among actinomycetes; due to the similarity of their chemical structure, they are similar in their biological action, regulating morphogenesis and biosynthesis of secondary metabolites in representatives of different actinomycete species [4–8]. The test strains used to detect A-factor-group regulators are spontaneous or induced mutants of actinomycetes that fail to produce these regulators and, consequently, are unable to form aerial mycelium and spores. The test strains respond to the presence of A-factor-group regulators in the medium by regaining their capacity for morphogenesis and biosynthesis of secondary metabolites. In experiments with A-factor-group regulators produced by different species, these regulators were shown to exhibit a cross effect [4].

The substances of the A-factor group are endogenous autoregulators. The effect of A-factor is observed at a concentration as low as 1.2×10^{-12} M; hence, it is among substances producing the strongest biological action [1]. Like the majority of the species studied, *S. griseus* exhibits a high level of A-factor biosynthesis and secretion: its diffusion area exceeds the size of the colony that produces the regulator by several times [7]. We have proposed that the action of A-factor-group regulators can be polymodal; namely, at high concentrations they may have another regulatory effect at the population level. For example, these factors may influence spore germination. Therefore, in this study, we aimed to determine the effect of A-factor on spore germination, the earliest stage of the actinomycete life cycle. Representatives of three actinomycete species, differing in the ability to synthesize regulators of the A-factor group, were the subjects of this study.

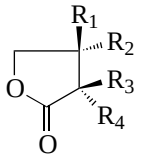
MATERIALS AND METHODS

The subjects of this study were representatives of three *Streptomyces* species: *S. griseus* 773 (=INA 00987), *S. coelicolor* A3(2), and *S. avermitilis* JCM5070. Strain *S. griseus* 1439 (=INA 00891) served as a test organism which responded to the presence in the medium of regulators of the A-factor group by aerial mycelium formation [11].

Actinomycetes were grown at 28°C on agarized soybean medium containing 1% glucose, 2% soybean flour, 0.5% NaCl, and 2% agar in tap water (pH 6.9–7.0).

[1] Corresponding author. E-mail: instra@online.ru

0026-2617/03/7206-0682$25.00



Regulators	R_1	R_2	R_3	R_4
A-factor	—H	—CH_2OH	—CO—$(CH_2)_4$—CH(CH_3)(CH_3)	—H
Homolog of A-factor	—H	—CH_2OH	—CO—$(CH_2)_3$—CH(CH_3)(CH_3)	—H
Homolog of A-factor	—H	—CH_2OH	—CO—$(CH_2)_2$—CH(CH_3)(CH_3)	—H
Acl-1a	—H	—CH_2OH	—CHOH—$(CH_2)_4$—CH(CH_3)(C_2H_5)	—H
Acl-1b	—H	—CH_2OH	—H	—CHOH—$(CH_2)_4$—CH(CH_3)(C_2H_5)
Acl-2a	—H	—CH_2OH	—CHOH—$(CH_2)_4$—CH(CH_3)(CH_3)	—H
Acl-2b	—H	—CH_2OH	—H	—CHOH—$(CH_2)_4$—CH(CH_3)(CH_3)
Acl-2c	—H	—CH_2OH	—CHOH—$(CH_2)_3$—CH(CH_3)(C_2H_5)	—H
Acl-2d	—CH_2OH	—H	—CHOH—$(CH_2)_4$—CH(CH_3)(CH_3)	—H

Fig. 1. Chemical structure of A-factor, its homologs, and Acl-factors [1, 4].

The A-factor used in this work was synthesized in 1982 in the laboratory of A.S. Khokhlov, Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences. The stock solution of A-factor (500 μg/ml) was sterilized by passing through a Zeitz filter.

The strains were grown for 14 days on soybean agar, after which the spores were washed off with distilled water and filtered through a cotton filter. The suspension titers were determined in Goryaev's chamber. Before plating onto the surface of agarized medium, the suspensions were diluted so that the expected SFU number ranged from 50 to 500 colonies per dish. The colonies were counted after seven days of growth.

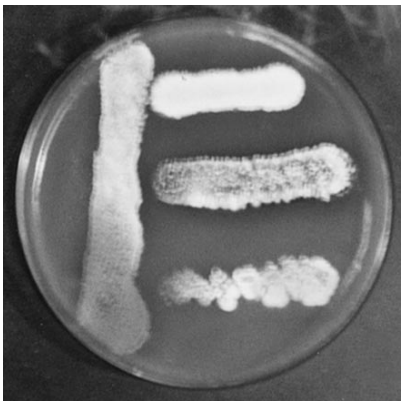


Fig. 2. Induction of aerial mycelium formation in the test strain *S. griseus* 1439, which responds to the presence of A-factor-group regulators in the medium. The following strains were plated by horizontal streaks (from top to bottom): *S. griseus* 773, *S. coelicolor* A3(2), *S. avermitilis* JCM5070.

The results were processed using the methods of mathematical statistics [12].

RESULTS AND DISCUSSION

The biological action of A-factor was previously judged from the induction or stimulation of morphogenesis and biosynthesis of secondary metabolites [4]. The effect of A-factor on actinomycete spore germination has never been studied. We expected that A-factor might have a selective effect on actinomycete spore germination. Based on the previous data, we used two actinomycete strains which differed in their ability to form regulators of the A-factor group: *S. griseus* 773, a producer of A-factor and two of its lower homologs [4, 13], and *S. coelicolor* A3(2), which produces six Acl-factors, A-factor analogues [4, 14] (Fig. 1). The strain *S. avermitilis* JCM5070 does not produce regulators of the A-factor group, which was confirmed when the test strain *S. griseus* 1439 and *S. avermitilis* JCM5070 were grown together on agarized medium. Unlike the streptomycete strains capable of producing regulators of the A-factor group (Fig. 2), *S. avermitilis* JCM5070 failed to induce spore formation of the test strain.

After plating spore suspensions of three streptomycete strains on agarized medium, normal colony development was followed by aerial mycelium and spore formation in all cases, suggesting that all three strains develop normally without A-factor addition.

However, after the introduction of A-factor into the medium (0.01–10 μg/ml), the number of colonies exhibited variations. Note that in the control and experimental variants plating was performed from the same suspensions; i.e., the number of the spores plated was the same in control and experimental variants. The table presents the results obtained with and without the addition of A-factor to the solid medium. As can be seen from the table, at certain concentrations of A-factor in solid medium the number of germinated spores could increase by up to 70%.

The above effect depended on the A-factor concentration in the medium. In strain *S. griseus* 773, the number of colonies in the experiment differed significantly from that in the control at an A-factor concentration of 10 μg/ml; lower concentrations caused no increase in the number of germinated spores (grown colonies) compared to the control. In strain *S. coelicolor* A3(2), stimulation of germination was observed at an A-factor concentration equal to 1 μg/ml; lower and higher concentrations were inefficient.

When previously studying a collection of asporogenic, nocardia-like, and oligosporous morphological variants of 28 strains of 23 streptomycete species [15], we showed that different strains needed different amounts of A-factor in the medium to produce aerial mycelium, spores, and pigments. For example, restoration of spore formation in the asporogenic variant of *S. griseus* K3826 was observed at 10 μg/ml A-factor in the medium, whereas a concentration of 1 μg/ml was sufficient for the two other A-factor-dependent variants, *"S. citreofluorescens"* K3506 and *"S. viridovulgaris* subsp. *albomarinus"* K3865. In our other studies, the biosynthesis of antibiotics was also shown to depend on A-factor concentration, namely, the biosynthesis of valinomycin in *S. cyaneofuscatus* and the biosynthesis of rifampicin in *Amycolatopsis mediterranei* [5, 10].

Another conclusion that can be inferred from our results is that A-factor stimulates spore germination of only those strains that synthesize regulators of the

CFU numbers of streptomycetes (million/ml of suspension) revealed at different concentrations of A-factor in solid medium

Species, strain	Ability to produce regulators of the A-factor group	Amount of A-factor added to the medium (μg/ml)				
		0	0.01	0.1	1	10
S. griseus 773	A-factor and 2 its homologs	36.4 ± 4.9	34.3 ± 4.6	37.2 ± 4.9	30.1 ± 4.4	60.7 ± 10.5*
S. coelicolor A3(2)	6 Acl-factors	8.0 ± 1.5	8.8 ± 1.2	8.0 ± 0.9	14.0 ± 1.9*	7.8 ± 0.5
S. avermitilis JCM5070	Not produced	54.0 ± 13.0	56.0 ± 8.5	55.0 ± 5.0	48.0 ± 8.4	54.0 ± 6.3

* The value exceeded the control one at a significance level of 0.95.

A-factor group. Under the conditions of our experiments, A-factor addition had no effect on spore germination in *S. avermitilis* JCM5070. All platings were performed four times, and the significance level of the data was > 0.95.

Thus, a new regulatory function of the A-factor has been discovered. It stimulates spore germination in streptomycetes, which results in an increased number of grown colonies. The spores are normally heterogeneous, which manifests itself in their asynchronous germination. The biological implication of this phenomenon seems to lie in the fact that under unfavorable conditions germinated spores may perish, whereas the spores that are viable but do not germinate can save the population. The presence of A-factor suggests the development of an actinomycete colony, indicative of favorable growth conditions and is thus a signal for the majority of spores to germinate.

ACKNOWLEDGMENTS

We are grateful to V.D. Kuznetsov for helpful discussions.

REFERENCES

1. Khokhlov, A.S., *Nizkomolekulyarnye mikrobnye autoregulyatory* (Low-Molecular-Weight Microbial Autoregulators), Moscow: Nauka, 1988.
2. Khokhlov, A.S., *Microbial Autoregulators*, Harwood: Academic, 1991.
3. Khokhlov, A.S., Tovarova, I.N., Borisova, L.N., Pliner, S.A., Shevchenko, L.A., Kornitskaya, E.Ya., Ivkina, N.S., and Ropoport, I.A., A-Factor Promoting Streptomycin Biosynthesis by a Mutant Strain of *Actinomyces streptomycini*, *Dokl. Akad. Nauk SSSR*, 1967, vol. 177, no. 1, pp. 232–235.
4. Efremenkova, O.V., Anisova, L.N., and Bartoshevich, Yu.E., Regulators of the Differentiation in Actinomycetes (A Review), *Antibiot. Med. Biotekhnol.*, 1985, no. 9, pp. 687–707.
5. Anisova, L.N., Blinova, I.N., Efremenkova, O.V., Smirnova, G.M., and Khokhlov, A.S., Differentiation Regulators in *Streptomyces cyaneofuscatus*, *Mikrobiologiya*, 1984, vol. 53, pp. 890–895.
6. Kornitskaya, E.Ya., Tovarova, I.I., and Khokhlov, A.S., A Study of the Production of A-Factor by Various Actinomycetes, *Mikrobiologiya*, 1976, vol. 45, pp. 302–305.
7. Efremenkova, O.V., Anisova, L.N., and Khokhlov, A.S., Excretion by Various Actinomycetes of Compounds Inducing Sporulation in an Asporogenous Mutant of *Streptomyces griseus*, *Mikrobiologiya*, 1979, vol. 48, pp. 999–1003.
8. Eritt, I., Gräfe, U., and Fleck, W.F., A Screening Method for Autoregulators of Anthracycline-Producing Streptomycetes, *Z. Allg. Microbiol.*, 1982, vol. 22, pp. 91–96.
9. Efremenkova, O.V., Anisova, L.N., Kamzolkina, O.V., Dmitrieva, S.V., Gorin, S.E., and Bartoshevich, Yu.E., Production of A-Factor-Type Regulators by representatives of the genus *Micromonospora*, *Antibiot. Med. Biotekhnol.*, 1987, vol. 32, no. 9, pp. 643–648.
10. Bushueva, O.A., Efremenkova, O.V., Gorin, S.E., and Bartoshevich, Yu.E., The Effect of Low-Molecular-Weight Regulators on the Biosynthesis of Rifamycin B by *Amycolatopsis mediterranei* Strains, *Antibiot. Khimioter.*, 1991, vol. 36, no. 3, pp. 11–15.
11. Borisova, L.N., Ivkina, N.S., and Gomenyuk, L.A., A Study of Complementary Combinations of Secondary Biosynthetic Mutants of *Actinomyces streptomycini*, *Dokl. Akad. Nauk SSSR*, 1970, vol. 192, pp. 199–202.
12. Lakin, G.F., *Biometriya* (Biometrics), Moscow: Nauchnaya Shkola, 1990.
13. Kleiner, E.N., Pliner, S.A., Soifer, V.S., Onoprienko, V.V., Balashova, T.A., Rozynov, B.V., and Khokhlov, A.S., Structure of A-Factor, a Bioregulator from *Streptomyces griseus*, *Bioorg. Khim.*, 1976, vol. 2, pp. 1142–1147.
14. Anisova, L.N., Blinova, I.N., Efremenkova, O.V., Koz'min, Yu.P., Onoprienko, V.V., Smirnova, G.N., and Khokhlov, A.S., Regulators of the Development of *Streptomyces coelicolor* A3(2), *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1984, pp. 98–108.
15. Efremenkova, O.V., Gruzina, V.D., Sumarukova, I.G., and Kuznetsov, V.D., Search for A-Factor–Dependent Variants in Actinomycete Populations, *Mikrobiologiya*, 2003, vol. 72, no. 6, pp. 766–779.