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EXPERIMENTAL ARTICLES

Search for A-factor-Dependent Variants in Actinomycete Populations

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Abstract—A study of 28 nocardia-like, asporogenous, and oligosporous spontaneous morphological variants belonging to 23 species of streptomycetes revealed five strains producing regulators of the A-factor group. *Streptomyces griseus* 1439, which forms aerial mycelium and spores only in the presence of exogenous A-factor, was used as the test strain. Among the 28 spontaneous variants, three new A-factor-dependent strains were revealed, which represented the species *Streptomyces griseus*, "*S. citreofluorescens*," and "*S. viridovulgaris* subsp. *albomarinus.*" These weakly differentiated variants did not produce A-factor and behaved as its recipients, responding by changes in their morphological characteristics at a concentration of this regulator in the medium of 0.01 μg/ml or higher. The original collection strains in whose populations the variants were selected produced substances of the A-factor group. The A-factor-dependent variants differed in the level of the regulator required for maximal expression of the morphological characteristics: it was necessary to introduce the A-factor at a concentration of 1 μg/ml for "*S. citreofluorescens*" and "*S. viridovulgaris* subsp. *albomarinus*" and at 10 μg/ml for *S. griseus.*

Key words: A-factor, regulators of the A-factor group, actinomycetes, streptomycetes, cytodifferentiation, A-factor-dependent variants.

A-factor is an endogenous regulator of cytodifferentiation in *Streptomyces griseus*, controlling morphogenesis and biosynthesis of streptomycin [1–3]. In addition to A-factor, actinomycetes are known to synthesize a whole group of substances that are homologs and close analogues of A-factor [4]. For example, *S. coelicolor* A3(2) does not produce A-factor but forms no less than six compounds with the same function, called Acl factors [5]. The biological action of these compounds is similar to that of A-factor; therefore, the term *A-factor* is often used in a broad sense, instead of the term *regulators of the A-factor group*.

At present, much attention is being given to the molecular mechanisms of the action of specific microbial regulators [6–8]. Works concerned with the study of the A-factor-group regulators are based on the asporogenous and nocardia-like *S. griseus* variants obtained earlier. Under the influence of the A-factor released into the medium by the original strain, the nocardia-like mutant *S. griseus* 1439 (=INA 00891) forms aerial mycelium and spores and synthesizes streptomycin. A-factor was first discovered, isolated, and identified with the use of this strain [9]. Similar indicator strains of *S. griseus* were used in the works of other authors [10, 11]. Apart from the *S. griseus* variants, A-factor-dependent strains of *S. bikiniensis* and *S. cyaneofuscatus* were also used as indicators [12, 13]. It was shown by using them that the A-factor-group regulators are widespread among actinomycetes of different systematic groups [3, 12, 14].

In the population of *S. griseus*, morphogenetic impairments in most nocardia-like and asporogenous variants are caused by loss of the ability to synthesize A-factor. More than 90% of these variants are A-factor-dependent: when cultivated in medium with A-factor they restore the phenotype of the initial strain, undergo a normal developmental cycle, and form aerial mycelium and spores [11]. However, it was not clear whether this regularity is specific for the species *S. griseus* or is widespread in the streptomycetes of other species.

The aim of this work was to reveal, among 28 spontaneous weakly differentiated variants of 23 species of streptomycetes, A-factor-dependent strains in which, as in *S. griseus*, the impairment of differentiation is due to blockage of the synthesis of the A-factor-group regulators.

MATERIALS AND METHODS

This work used oligosporous, nocardia-like, and asporogenous variants of actinomycetes from the collection of the Laboratory of Classification and Storage

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Table 1. Study of the ability to form/perceive A-factor in morphological variants of actinomycetes cocultivated with the test strain *S. griseus* 1439

Species	Morphological variants			
	Strain	Morphological variant*	Production of the A-factor-group regulators	Ability to respond to A-factor**
Saccharopolyspora erythraea (=Streptomyces erythreus)	K3637	NL	–	–
	K3077	OS	–	–
Streptomyces abikoensis	K3473	NL	–	–
S. albogriseolus	K4062	NL	–	–
"*S. albus* var. *fungatus*"	K3154	NL	–	–
S. aureoverticillatus	K3795	OS	–	–
S. chattanoogensis	K1019	NL	–	–
S. chrysomallus	K3209b	OS	+	–
	K3209zh	OS	+	–
	K3508	OS	+	–
"*S. citreofluorescens*"	K3506	OS	–	+
S. fluorescens	K3505	NL	–	–
S. fradiae	K3085	NL	–	–
S. griseus	K3628	NL	+	–
	K3826	AS	–	+
	K3883	NL	+	–
S. kanamyceticus	K3627	OS	–	–
"*S. lavendulae-grasserius*"	K3603	NL	–	–
"*S. moderatus*"	K3910	NL	–	–
"*S. olivobrunneus*"	K3884	NL	–	–
"*S. orientalis*"	K3759	NL	–	–
S. parvulus	K3849	NL	–	–
S. rimosus	K3878	OS	–	–
S. roseolus	K3876	NL	–	–
S. spheroides	K3790	NL	–	–
"*S. tumemacerans*"	K3608	NL	–	–
"*S. viridovulgaris* subsp. *albomarinus*"	K3865	AS	–	+
Streptomyces sp.	K3761	NL	–	–

* Morphological variants: OS, oligosporous; AS, asporogenous; NL, nocardia-like.

** The character of growth in medium containing A-factor at a concentration of 10 μg/ml: "+" denotes occurrence of changes in morphological characteristics (formation of aerial mycelium and sporogenesis); "–" means that no changes in morphological characteristics were observed.

of Unique Microorganisms, Institute of Microbiology, Russian Academy of Sciences (Table1). The test strain for determination of the content of A-factor-group regulators in the medium—*Streptomyces griseus* 1439 (=INA 00891)—was obtained by Borisova *et al.* [2].

Submerged cultivation of actinomycetes was carried out aerobically on a shaker (220 rpm) at 28°C in Soybean fermentation medium containing (%) glucose, 4; soybean flour, 3; $(NH_4)_2SO_4$, 0.6; KH_2PO_4, 0.05; NaCl, 0.25; and chalk, 0.6. A solid medium soybean agar was used, which contained 1% glucose, 2% soybean flour, 0.5% NaCl, and 2% agar in tap water (pH 6.9 to 7.0).

The A-factor used in this work was synthesized in 1982 in the laboratory headed by A.S. Khokhlov (Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences).

Table 2. Characterization of the A-factor-dependent morphological variants

Species, strain	Minimal effective concentration of A-factor in the medium, μg/ml	Concentration of A-factor in the medium causing maximum effect, μg/ml
S. griseus K3826	0.01	10
"*S. citreofluorescens*" K3506	0.01	1
"*S. viridovulgaris* subsp. a*lbomarinus*" K3865	0.01	1

RESULTS AND DISCUSSION

The collection consisting of 28 weakly differentiated spontaneous variants of 23 species of streptomycetes used in the work was represented by the oligosporous, asporogenous, and nocardia-like morphological types [15, 16]. The affiliation of the variants with one of these morphological types is shown in Table 1.

The production of A-factor group-regulators by different cultures of actinomycetes was judged from changes in the morphological characteristics in the test strain *S. griseus* 1439 [4]. For this purpose, the surface of the agar medium was streaked with two-day submerged cultures of the actinomycete studied and the test strain. The streaks were spaced 5 to 10 mm apart. The release of an A-factor-group regulator in the medium by the actinomycete studied was evidenced by the formation of aerial mycelium by the test strain on the 2nd–30th day of growth (Table 1).

This test showed that only five of the 28 cultures synthesized substances of the A-factor group. These were three *"S. chrysomallus"* strains (K3209b, K3209zh, and K3508) and two *S. griseus* strains (K3628 and K3883). No changes in their development were observed when they were grown in medium containing exogenously added synthetic A-factor.

To search for A-factor-dependent variants, agar media containing A-factor at a concentration from 0.001 to 50 μg/ml were inoculated with two-day submerged cultures of actinomycetes.

Among the 23 cultures not forming substances of the A-factor group, three cultures responded by changing their properties when this regulator was introduced into the medium; i.e., they were A-factor-dependent (Table 1); these were strains of *S. griseus*, *"S. citreofluorescens,"* and *"S. viridovulgaris* subsp. *albomarinus."* As shown by cocultivation with the test organism *S. griseus* 1439, these weakly differentiated variants (K3826, K3506, and K3865) do not form A-factor (or A-factor-like substances), as distinct from the initial collection strains *S. griseus* INA 00988, *"S. citreofluorescens"* RIA 1187, and *"S. viridovulgaris* subsp. *albomarinus"* RIA 1512. The characterization of the dependence of the three weakly differentiated variants on A-factor is given in Table 2. It follows from the results obtained that the minimal concentrations of A-factor in the medium required for the induction of cytodifferentiation were the same for all three A-factor-dependent strains. At the same time, these variants differed in the level of exogenously introduced A-factor that was required for maximum manifestation of the morphological changes (the intensity of the development of aerial mycelium, spore formation, and the release of soluble pigment).

Thus, in the spontaneous variants K3826, K3506, and K3865, as in the spontaneous and induced mutants of *S griseus*, *S. bikiniensis*, and *S. cyaneofuscatus* described earlier [1, 12, 13, 17], the morphogenesis impairments in the course of the life cycle are caused by loss of the ability to synthesize A-factor-group regulators. This is an example demonstrating the applicability of the Vavilov's law of homologous series to actinomycetes [16]: as a result of impairment of biosynthesis of endogenous regulators of the A-factor group, the representatives of minor morphological types are formed in the populations of streptomycetes of different species.

When actinomycete cultures are isolated from natural sources, for example, from soil samples, nocardia-like or asporogenous variants are infrequently isolated, for which generic affiliation is difficult to establish. If these variants are A-factor-dependent, it may be expedient for their rapid generic identification to plate them onto medium containing A-factor or culture liquid of a strain producing A-factor-group regulators. Such strains include *S. griseus* INA 00987, *S. coelicolor* A3(2), and others [3, 14]. The following method is also possible: close streaking of agar medium with a strain forming A-factor-group substances as well as the isolate supposed to be an A-factor recipient, which is then able to respond to the presence of A-factor in the medium by forming aerial mycelium, spores, and pigment.

When approving the latter method, the three A-factor-dependent variants used in this work responded in the same way to the presence of A-factor in the medium. When agar medium was inoculated with each of them in pairs with the strains listed in Table 1, the ability to synthesize A-factor-group substances was revealed in the same five (of 28) variants that were discovered in the first part of this work (when the test strain *S. griseus* 1439 was used).

It should be noted that biological methods sometimes do not allow the A-factor producers to be revealed, since either the isolate studied or the test culture can inhibit each other due to the synthesis of antibiotics by them. In other cases, the analysis may be difficult because the media that are most favorable for growth and cytodifferentiation are different. In this connection, the expansion of the range of the revealed and characterized A-factor-dependent variants of acti-

nomycetes will alleviate the choice of appropriate test strains for the search for organisms synthesizing A-factor-group regulators among representatives of different species of actinomycetes.

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